2020-08

The distribution, control techniques and impact of Parthenium hysterophorus on flower visitors and soil chemical properties in Meru district, Tanzania

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THE DISTRIBUTION, CONTROL TECHNIQUES AND IMPACT OF *Parthenium hysterophorus* ON FLOWER VISITORS AND SOIL CHEMICAL PROPERTIES IN MERU DISTRICT, TANZANIA

Fredrick Ojija

A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

August, 2020

ABSTRACT

In Tanzania, the invasive plant *Parthenium hysterophorus* threatens natural and semi–natural environments. Little is known about how this species affects plant–pollinator interactions and soil–chemical properties. Roadside survey was conducted to assess *P. hysterophorus* invasion status within and outside Arusha National Park (ANP), Tanzania. Soil samples were also collected in invaded and uninvaded plots to investigate the impact of *P. hysterophorus* on soil–chemical properties. To test alternative natural–based management strategies, the study examined the bio–herbicide potential of *Desmodium uncinatum* leaf (DuL) crude extract and the competitive ability of fodder legume plant species (*Lablab purpureus*, *Desmodium intortum* and *Medicago sativa*) to suppress *P. hysterophorus* in pot and plot experiments. Results showed that ANP is currently uninvaded with *P. hysterophorus*, but some adjacent villages i.e. King'ori, Maleu, Napoco, Ngongongare, Ngurdoto, Oligilai and Sakila have already invaded by this invasive. *Parthenium hysterophorus* invaded site was more acidic with lower electrical conductivity, less calcium and phosphorus, and high cation exchange capacity. The DuL extract suppressed *P. hysterophorus* growth vigour, particularly at higher concentrations (>70%). At these concentrations, the invasive seedling stem height was >30% shorter, and seed germination was inhibited by >55% compared to seedlings sprayed with lower concentrations. When *P. hysterophorus* was grown in combination with all three test plants, its seedling stem heights and total fresh biomass were reduced by >60% and >59% in pots, and >40% and >45% in field plots respectively, compared to when grown alone or in mixture with just *D. intortum* or *M. sativa*.

Moreover, surveys conducted on invaded and uninvaded sites to investigate the impact of *P. hysterophorus* on plant–pollinator interactions when two common target plants (*Ocimum gratissimum* and *Ageratum conyzoides*) were present showed that flower visitation rate to target plants was significantly lower in invaded plots than in uninvaded plots. This implies that *P. hysterophorus* may be disrupting pollen flow. The study recommends that the use of bio–herbicide and suppressive plant species to control the invasive should be promoted. However, it emphasizes the use of native suppressive plant species because non–natives may turn into invasives in the future.

DECLARATION

I, Fredrick Ojija hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

Fredrick Ojija **Signature:** **Date:**
Canditate

Pro. Anna Treydte **Signature:** **Date:**

Dr. Sarah Anold **Signature:** S. Arnold **Date:**

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CERTIFICATION

The undersigned certify that they have read the dissertation titled "Assessing the spread, management and impact of *Parthenium hysterophorus* on flower visitors and soil chemical properties in Tanzania" and recommended for examination in fulfilment of the requirements for the degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology.

Pro. Anna Treydte **Signature:** **Date:**

Dr. Sarah Anold **Signature:** S. Anold **Date:**

ACKNOWLEDGEMENTS

First and foremost, I thank the almighty God for giving me the strength and good health to complete this research wok.

I am extremely thankful to my supervisors, Prof. A. C. Treydte from the Nelson Mandela Institution of Science and Technology (NM–AIST) and Dr. S. E. J. Arnold from Natural Resources Institute, University of Greenwich for their vital help, advice, moral support and guidance throughout this study. I frankly again thank them for their knowledgeable suggestions and efforts which have contributed positively to the completion of this dissertation.

Thanks are due to the Tanzania Commission for Science and Technology (COSTECH), Tanzania National Park Authority (TANAPA) and Tanzania Wildlife Research Institute (TAWIRI) for providing a permit to conduct field surveys in Arusha National Park (ANP).

My sincere gratitude to the World Bank through its African Centre of Excellence, "Centre for Research, Agricultural advancement, Teaching Excellence and Sustainability in Food and Nutritional Security (CREATES)", in the School of Life Sciences and Bioengineering at the Nelson Mandela Institution of Science and Technology (NM–AIST) in Arusha, Tanzania for funding this study. I am also grateful for the Idea Wild for supporting the study through research equipment.

Thanks are also due to Mr. R. Kilewa from Agricultural Division at Tropical Pesticide Research Institute for providing *Parthenium hysterophorus* seeds, Dr. P. Venkataramana in the Department of Sustainable Agriculture, Biodiversity and Ecosystem Management at NM–IST for providing *L. purpureus* seeds and Mrs. C. Manase an ecologist from ANP for preparing rainfall and vehicle data. Further, I would like to acknowledge village leaders, local people, friends, field assistants and laboratory technicians within and outsides NM–AIST who facilitated me during my field surveys and experiments and thus, to complete this research work.

I would like to acknowledge and express my sincere appreciation to my parents and relatives for their financial and in–kind support. Last but not the least, I thank my Wife Eng. L. P. Lutambi, my sons Ferdinand, Ferdison and Fredy for giving me time to study and thus, denied their time; your patience, kindness and love made me to complete this study successfully.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
ADB	Aboveground Dry Biomass
AFB	Aboveground Fresh Biomass
ANP	Arusha National Park
AU$	Australian Dollar
BDB	Belowground Dry Biomass
BFB	Belowgrond Fresh Biomass
C	Carbon
ca.	Circa (meaning approximately)
Ca	Calcium
CABI	Centre for Agriculture and Bioscience International
CBC	Classical Biological Control
Chl	Chlorophyll
CEC	Cation Exchange Capacity
cm	Centimeter
CO_2	Carbon dioxide
CREATES	Centre for Research, Agricultural Advancement, Teaching Excellence and Sustainability
^0C	Degree centigratde
DMSO	Dimethyl Sulphoxide
DuL	*Desmodium uncinatum* Leaf
DTPA	Diethylene Triamine Pentaacetic Acid
EC	Electrical Conductivity
FAO	Food and Agriculture Organization
g	Gram
GISP	Global Invasive Species Programme
ha	Hectre
h	Hour
H	Hydrogen
IAPs	Invasive Alien Plant Species

IIBC	International Institute of Biological Control
IP	Inhibition Percentage
IUCN	International Union for Conservation of Nature
K	Potassium
km	Kilometer
l	Litre
m	Meter
m.a.s.l	Meters Above Sea Level
mm	Millimetre
Mg	Magnesium
Mn	Manganese
N	Nitrogen
Na	Sodium
nm	Nanometer
NM–AIST	Nelson Mandela Institution of Science and Technology
O	Oxygen
OC	Organic Carbon
OD	Optical Density
P	Phosphorus
RCI	Relative Competition Intensity
RII	Relative Interaction Intensity
SD	Standard Deviation
SE	Standard Error
TANAPA	Tanzania National Park Authority
TAWIRI	Tanzania Wildlife Research Institute
TPRI	Tropical Pesticides Research Institute
UDSM	University of Dar es Salaam
UNEP	United Nation Environmental Programme
US$	United States Dollar
USA	United States of America

CHAPTER ONE

INTRODUCTION

1.1 Background of the Problem

Human activities and increased movement of people and goods across continents or countries due to globalization have immensely enhanced the introduction and long–distance dispersal of alien species into new environments (Axmacher & Sang, 2013; Dukes & Mooney, 1999; Early *et al*., 2016; Pratt *et al*., 2017; Primack, 2010; Shackleton *et al*., 2017). Climate change along with anthropogenic activities also accelerate dispersal of non–native species outside their natural range (Axmacher & Sang, 2013; Brunel *et al*., 2014; Dukes & Mooney, 1999; Nigatu *et al*., 2010; Taylor *et al*., 2012). According to the "tens rule" of Williamson, only 10% of transported species are released into novel locations, 10% of these introduced species successfully establish new populations, and 10% of these established individuals expand their geographical location and become invasive (Lake & Leishman, 2004; Primack, 2010). Invasive species generally expand their population from the location of original arrival into natural or semi–natural vegetation communities (Brunel *et al*., 2014; Early *et al*., 2016; Primack, 2010). Therefore, biological invasion occurs when accidentally or intentionally, a non–native or alien plant species is introduced into new habitats where it proliferates, colonize and become invasive (Axmacher & Sang, 2013; CBD, 2002; Ellison & Cock, 2017; Foxcroft *et al.*, 2019; Pratt *et al.*, 2017). Biological invasion is an element of human–induced global change, along with habitat disturbances (Bellingham *et al*., 2018; Lake & Leishman, 2004; Shackleton *et al*., 2017). Too often, plant invasions cause homogenization of natural flora community in the recipient ecosystems (Bellingham *et al*., 2018; Hejda *et al*., 2009; Lake & Leishman, 2004).

The terms 'alien', 'non-native', and 'introduced' allude to those species or taxa that may have crossed an international border and are considered as 'foreign' or 'new' to a place where they had never existed or lived before (Obiri, 2011; Perkins *et al*., 2011; Pyšek *et al*., 2004; Richardson *et al*., 2000). Many of these species fail to establish self–sustaining populations in their novel locations (as in the tens rule of Williamson). However, sometimes when a species relocates to a new site its population grow exponentially due to lack of natural enemies i.e. potential pests, pathogens and predators that control the species within its home range (Cappuccino & Carpenter, 2005; Pyšek *et al.*, 2004; Richardson *et al.*, 2000). Alien

aggressive plant species are usually known as invasive alien plant species (IAPs) or invaders (Pyšek *et al*., 2004).

Alien invasive species exert environmental or economic harm, and negatively interfere with human wellbeing (Adkins & Shabbir, 2014; Bajwa *et al*., 2019; Tanveer *et al*., 2015; Witt *et al.*, 2018). Thus, IAPs may be defined as non–native plant species, which threaten global biodiversity, ecosystem function, services and structure, agricultural production, economy and the sustainability of human societies (Axmacher & Sang, 2013; Ellison & Cock, 2017; Hinz & Schwarzlaender, 2004; Perkins *et al*., 2011). The International Union for Conservation of Nature (IUCN) defines invasive species as animals, plants or other living organisms introduced into a new range where they establish and pose negative impacts on the recipient environments and native species (IUCN, 2000). The process by which an invasive species enters into new environment, its establishment and eventual spread in other habitats is known as invasion (Richardson *et al*., 2000; Richardson & Rejmánek, 2011). Invasibility implies the level of how vulnerable an environment is to invasions from IAPs (Foxcroft *et al.*, 2010; Perkins *et al.*, 2011), whereas invasiveness is the ability of alien species to establish self–sustaining populations and to expand in a natural vegetation community, with which they had not existed before (Perkins *et al*., 2011; Pyšek *et al*., 2004; Richardson & Rejmánek, 2011).

Invasive plants are considered competitively superior to native plant species (Čuda *et al*., 2015; Vilà & Weiner, 2004). They have strategies or specific suites of traits that influence their invasive potential, i.e. enabling them to colonize their novel environments and compete with native species (Perkins *et al*., 2011). These include capacity to produce abundant seeds (Kaur *et al*., 2014), rapid germination and growth rate (Axmacher & Sang, 2013; Čuda *et al*., 2015), high survival rate, tall stature and early or late flowering (Čuda *et al*., 2015), short life cycle, many dispersal mechanisms (Adkins & Shabbir, 2014; Axmacher & Sang, 2013; Lurgi *et al.*, 2016; Qasem & Foy, 2001), ability to form persistent seed banks (Gioria *et al*., 2019) and long seed dormancy (Qasem & Foy, 2001). Their seeds also spread rapidly and establish along anthropogenic waterways and road networks (Axmacher & Sang, 2013; Christen & Matlack, 2006; Wabuyele *et al*., 2015). Further, IAPs are often free from biotic constraints outside their native range (Obiri, 2011; Perkins *et al*., 2011) as they lack effective natural enemies, for instance, bacteria, fungi, insects, mites, viruses and larger grazing animals (Hinz & Schwarzlaender, 2004; Lake & Leishman, 2004; Mitchell & Power, 2003). This may also

due to anti–microbial or anti–herbivory properties of IAPs (Cappuccino & Carpenter, 2005; Ellison & Cock, 2017; Mitchell & Power, 2003).

Further, IAPs exploit limiting resources more efficiently, and increase in biomass faster than native plant species (Bellingham *et al*., 2018; Rojas-Sandoval *et al*., 2016; Subhashni & Lalit, 2014). Following their establishment in the recipient ecosystem, IAPs displace native and valuable species through competition for resources such as light, nutrients, space, and water (Perkins *et al*., 2011; Pyšek *et al.*, 2004; Tanveer *et al.*, 2015; Vardien *et al.*, 2012). Many IAPs use allelopathy to suppress growth vigour, seed germination and development of native plant species by releasing allelochemicals into the environment (Callaway *et al*., 2008; Namkeleja *et al*., 2013). As a result, they alter vegetation structure of the recipient habitats by changing the diversity, richness and renewal of native flora and fauna species (Adkins & Shabbir, 2014; Bellingham *et al.*, 2018; Rojas-Sandoval *et al.*, 2016). Allelopathy can be defined as the adverse or favourable effects of one plant species on other plants through the release of allelochemicals or toxic compounds from living or dead plants (Bhadoria, 2010; Christina *et al*., 2015; Michael Van der Laan, 2007; Zhao *et al*., 2008). Plant species that have co–evolved or co–existed with allelopathic plants might be less susceptible to allelochemicals, whereas newly exposed plants such as natives could be sensitive to toxic compounds (Callaway & Aschehoug, 2000; Callaway *et al.*, 2008; Callaway & Ridenour, 2004; Cappuccino & Arnason, 2006). Thus, native plants without defensive mechanisms to counteract the allelochemicals released by the IAPs are more vulnerable to biological invasions (Callaway *et al.*, 2008).

Some invasive plants alter soil physico–chemical properties, nutrient cycles, shading, fire regimes, as well as reducing water infiltration (Fan *et al*., 2010; Osunkoya *et al*., 2017; Rojas-Sandoval *et al*., 2016; Vardien *et al*., 2012; Wakjira *et al*., 2009). They can deplete the soil seedbank of native species to the point that the latter cannot exert enough propagule pressure to occupy the bare space left following invasive removal (Gerber *et al*., 2008; Schuster *et al*., 2018). Furthermore, some invasive species negatively affect invertebrate and amphibian assemblages as well as species composition. For instance, Gerber *et al.* (2008) reported that invasive knotweeds (*Fallopia* spp.) in temperate riparian habitats (i.e., in Southern and Western Switzerland, South–western Germany and Eastern France) lowered the invertebrate abundance, biomass and morphospecies richness; and an invasive shrub, *Lonicera maackii* (Amur honeysuckle) lowered the species richness and evenness of native amphibians of the

August A. Busch Memorial Conservation Area in Missouri, USA (Watling *et al*., 2011). Moreover, IAPs have been found to disrupt inherent interactions such as pollination services among co–evolved native plant species in recipient environments which afterward may alter various ecosystem process (Aizen *et al.*, 2008, 2008; Albrecht *et al.*, 2014; Callaway *et al.*, 2008; Chittka & Schürkens, 2001; Traveset *et al.*, 2013). Pollination is a vital process that contributes to the production of food i.e. fruits and seeds (Barrios *et al*., 2016; Lázaro *et al*., 2013; Martins, 2014; Weissman & Schaefer, 2017). It maintains plant diversity and contributes towards biodiversity conservation and ecosystem health (Bjerknes *et al*., 2007; Martins, 2014; Stiers *et al*., 2014).

Biological invasions which disrupt pollination networks endanger plant community stability, and may lead to biodiversity loss or change in species composition (Knops *et al.*, 1999; Miranda *et al*., 2014; Tanveer *et al.*, 2015; Tracy *et al*., 2004). They may also impede smallholders' livelihoods and sustainable development, food security and nutrition (Foxcroft *et al*., 2010; Kaur *et al*., 2014; Richardson *et al.*, 2007; Richardson & Rejmánek, 2011). For instance, many crops grown by the world's poorest people (ca. 70% of the entire global population), often living in rural areas, are vulnerable to invasions, particularly in developing countries (Pratt *et al*., 2017). It is estimated by the United Nation Environmental Programme (UNEP) that IAPs cost the world economy an annual US $1.4 trillion (Obiri, 2011). Since invasion of IAPs is predicted to increase globally in many areas (Adhikari *et al*., 2015; Early *et al*., 2016; Kriticos *et al*., 2015; Mcconnachie *et al*., 2011) managing the spread of IAPs is crucial in order to ensure biodiversity and ecosystem conservation, human welfare, crop production, food security, poverty alleviation and overall economic growth (Crall *et al*., 2013; Maistrello *et al*., 2016; Pocock *et al*., 2017). Invasive plants also have some advantages such as provision of timber, firewood, charcoal and medicine (Dawson *et al.*, 2008; Mwendwa *et al.*, 2020). In general, biological invasion is one of the most important global change drivers of biodiversity loss after habitat loss (Nguyen *et al*., 2017).

1.1.1 Status of Invasive Species in Tanzania

The United Republic of Tanzania (URT), with a total area of 945 087 km², is located in eastern Africa between latitudes 1° 00' and 11° 45' south of the equator and longitudes 29° 15' and 41° 00' east of the Greenwich meridian. It is surrounded by Burundi, Democratic Republic of Congo, Kenya, Malawi, Mozambique, Rwanda, Uganda, Zambia and the Comoro Islands in the Indian Ocean (URT, 2014). It is one of the mega–biodiversity rich

countries on earth, supporting about 14 500 known species (URT, 2014). In addition to hosting six out of the 25 known biodiversity hotspots, Tanzania is among 25 countries with the highest number of endemic and threatened species (URT, 2014). In terms of numbers of large mammals, it accounts for 20% of Africa's large mammals, and more than one–third of all plant species in Africa (URT, 2014). Despite being a home to many animal and plant species, it also harbours many invasive plant species which threaten biodiversity conservation (Foxcroft *et al*., 2006; Ngondya *et al.*, 2016a; Witt *et al.*, 2018). Alien plants introduced for food, agroforestry and forestry are the most important cause for spreading of invasive species (Reichard & White, 2001; Richardson & Rejmánek, 2011). Trade of plants or their products also have played significant role in the spreading of alien plants in Tanzania (Dawson *et al*., 2008). Introductions of alien plants for ornamentation use are considered to be effective pathway for introducing invasive species (Reichard & White, 2001). For example, through the Amani botanical garden, several IAPs including *Maesopsis eminii* were introduced in Tanzania as an attempt to satisfy the social and economic needs of communities, for example, commercial gain such as timber production (Dawson *et al.*, 2008; Mwendwa *et al.*, 2020).

A survey conducted in Tanzania reported several invasive plant species with major threats to biodiversity, economy, environment and agricultural production (Witt *et al*., 2018). Other alien species such as *Argemone mexicana, Cedrela odorata, Eichhornia crassipes, Salvinia molesta, Opuntia monocantha, Setaria verticillata* that invaded Tanzanian ecosystems are described in literature such as Dawson *et al*. (2008), Foxcroft *et al*. (2006), Obiri (2011), Vardien *et al*. (2012), Ngondya *et al*. (2017) and Witt *et al*. (2018), in the Global Invasive Species Database (GISP), and in the Invasive Species Compendium of the Centre for Agriculture and Bioscience International (CABI). Alien species, which are considered non–invasive in the country today, can become invasive with disastrous effects in the future due to climate and anthropogenic changes (Moore *et al.*, 1987; Navie *et al*., 2005; Obiri, 2011; Subhashni & Lalit, 2014; Taylor *et al*., 2012; Thapa *et al*., 2018).

Among the IAPs listed in Table 1, *P. hysterophorus* (Plate 1) is considered as an invasive weed of global significance (Kaur *et al.*, 2014; Tanveer *et al*., 2015). In Tanzania, *P. hysterophorus* was first reported in the Arusha region in 2010 (Pratt *et al*., 2017). Since then it has spread in other four regions including Kilimanjaro, Manyara, Geita and Kagera (Fig.1). It is commonly known as carrot weed or 'gugu karoti' in Swahili. It has invaded perennial crops such as banana (*Musa acuminata),* tomatoes (*Solanum lycopersicum),* maize (*Zea*

mays), sunflower (*Helianthus annuus)* and common beans (*Phaseolus vulgaris)*, as well as pastures, grazing land, and roadsides (Personal observation, 2018). In addition to increasing smallholders' dependence on pesticides, *P. hysterophorus* threatens protected areas and livestock production. Because of this, urgent control is required to counteract its rapid invasion. The biology, ecology, life cycle, impact and global distribution of *P. hysterophorus* are elucidated in chapter two.

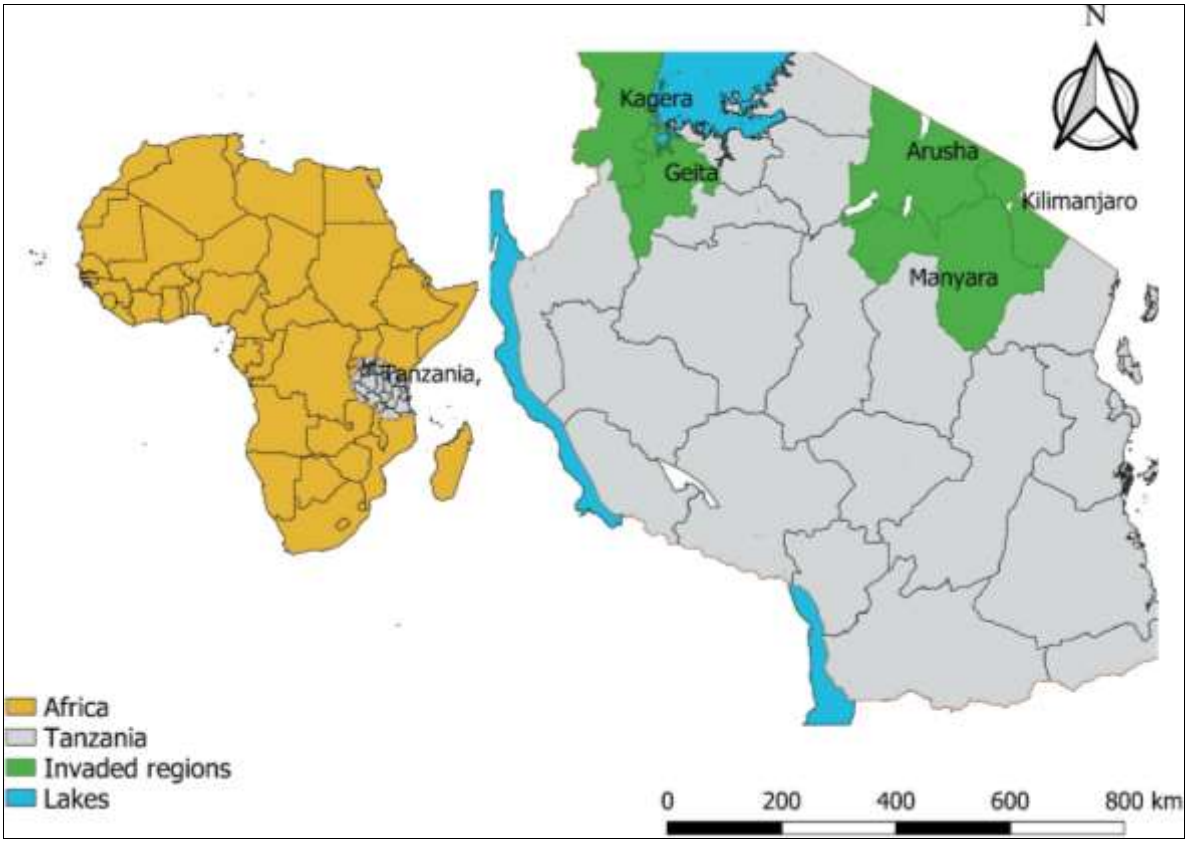


Figure 1: *Parthenium hysterophorus* Invaded Regions in Tanzania. Data from TPRI, 2018-2019

Table 1: Some of the Main Invasive Plant Species Found in Tanzania and their Negative Impacts on the Environment. Examples were Taken from Witt *et al*. (2018)

Invasive species	Family	Growth form	Impact
Acacia mearnsii	Fabaceae	Tree/shrub	Displaces/hinder growth of native species. Reduces surface water runoff and rangelands productivity. Increases soil nitrogen levels, and alter soil physical-chemical properties
Acacia melanoxylon	Fabaceae	Tree/shrub	Deleteriously affects biodiversity, displaces natural plant species, and alter soil nutrient as it increases levels of nitrogen
Agave angustifolia	Agavaceae	Shrub	Exploits resources (water and nutrients) more efficiently compared to natural vegetation, and displaces them in recipient ecosystems
Austrocylindropuntia subulata	Cactaceae	Shrub	Its dense thickets preclude animals' access to pastures and water resources. Also, its spines cause injuries to people and animals
Azadirachta indica	Meliaceae	Tree/shrub	Alter ecosystem vegetation structure, reduces the abundance of small mammals in coastal areas where it forms dense stands
Caesalpinia decapetala	Fabaceae	Climber/shrub	Detrimental to flora and fauna, climbs over vegetation in forest and woodland canopies. It forms impassable thickets inhibiting free movement of animals/people. Hampers forest management operations, as stems have large spines causing injuries to animals and people

Invasive species	Family	Growth form	Impact
Calotropis procera	Apocynaceae	Shrub	Displaces native species, forms huge and dense thickets, mainly along roadsides. Plant sap causes severe eye irritation, and illness when ingested
Cascabela thevetia	Apocynaceae	Tree or shrub	Very toxic, displaces native plant and animal species. It also forms dense thickets
Chromolaena odorata	Asteraceae	Shrub	Causes health problems to people and animals, reduces rangeland productivity, displaces native plants, changes vegetation community structure, and may increase fire intensities
Clidemia hirta	Melastomataceae	Shrub	Toxic to cattle, displaces native vegetation and threatens endemic/endangered species. It forms dense stands in recipient ecosystems
Datura stramonium	Solanaceae	Herb	Forms thick monospecific stands competing with and displacing native plants and crops. Toxic to both flora and fauna species
Lantana camara	Verbenaceae	Shrub	Affects biodiversity, hinder vegetation growth and reduces fodder production
Leucaena leucocephala	Fabaceae	Tree/shrub	It forms a large monospecific stands which outcompete native flora and fauna species, reduces habitats quality and alters secondary succession

Invasive species	Family	Growth form	Impact
			processes and ecosystem structure
Mimosa diplotricha	Fabaceae	Tree/shrub	It forms shades which prevent regeneration of light demanding flora species. Its dense stands hinder free movement of livestock and wildlife. It is also toxic to pigs and sheep
Opuntia stricta	Cactaceae	Succulent/shrub	Its spines cause injuries to people, livestock, and wildlife, prevents access to pasture. Displaces native flora and fauna species
Parthenium hysterophorus	Asteraceae	Herb	Noxious invasive weed, rapidly suppresses native vegetation through allelopathy and resource competition. Alters native plant community structure to monospecific stands, reduces rangeland productivity and crop yields, and causes health problems to people and animals.
Pinus patula	Pinaceae	Tree	Its dense stands displace native plant and animal species, and reduces water run-off
Pistia stratiotes	Araceae	Free-floating aquatic macrophyte	Hampers fishing activities, block water ways and slow water flow rates, destroy fish nesting sites, increases siltation rates and nutrient loading, decreases fish and macro-invertebrate survival

Invasive species	Family	Growth form	Impact
Prosopis juliflora	Fabaceae	Tree/shrub	Extirpates plants in invaded habitats, reduces rangelands and ecosystems grazing capacity, also decreases groundwater resource
Psidium guajava	Myrtaceae	Tree/shrub	Displaces native plant and animal species in invaded habitats. Adversely affects crops via allelopathy, and forms dense stands
Ricinis communis	Euphorbiaceae	Shrub	Forms thick stands which displace native plant species, particularly in riparian areas
Rubus niveus	Rosaceae	Climber/ shrub	Its dense thickets displace/hinder renewal of native species. It alters plants and ecosystems structure, hence threatening rare plant species
Senna spectabilis	Fabaceae	Tree	Outcompetes native plant species and impede their renewal. It grows rapidly and becomes dominant, also displaces fauna species
Solanum campylacanthum	Solanaceae	Shrub	Its dense stands reduce the abundance and diversity of native plant species. Animals, e.g. sheep may die if it consumes unripe fruits
Solanum mauritianum	Solanaceae	Tree/shrub	Toxic when eaten by animals, disrupts natural mechanisms of seed dispersal, and it displaces natural flora and fauna species
Tephrosia vogelli	Fabaceae	Tree/shrub	Toxic (leaves) to fishes, frogs, toads, molluscs, worms and insects. Also has allelopathy

Invasive species	Family	Growth form	Impact
Tithonia diversifolia	Asteraceae	Shrub	Reduces rangelands productivity, alters plant community structure, causes local extinction of some natives as it outcompetes vegetation and lowers their species diversity.
Xanthium strumarium	Asteraceae	Herb	Rapidly displaces other plants by forming large stands. Its toxicity causes death in livestock when consumed

(a)

(b)

Plate 1: *Parthenium hysterophorus*: (a) Seedling (left) and Mature Flowering Plant (right) and (b) Invaded Grazing Area in Arusha, Tanzania

1.1.2 Management of Invasive Species in Tanzania

In Tanzania, there are a number of laws, legislations and policies governing the introduction and management of alien plants. These include the Environmental Management Act No. 20 of 2004, the Plant Protection Act of 1997, the Forest Act No. 14 of 2002, National Fisheries Policy and Strategy Statement of 1998, Marine Parks and Reserves Act No 29 of 1994

Articles 10, Fisheries Act No 22 of 2003 section 22 (1) and the National Disaster Management Policy of 2004 that are administered by the Ministry of Environment and Natural Resources (Lyimo *et al*., 2009). The country is also a contracting part of the Nairobi protocol concerning protected areas and wild fauna and flora in the Eastern African region (21 June 1985). The protocol requires the parties to co–ordinate efforts and take vital actions to prohibit the accidental or deliberate introduction of harmful alien or new species to the East African region (art. 7) (Lyimo *et al*., 2009). The country is also a member of Forest Invasive Species Network for Africa. Forest Invasive Species Network for Africa was created in 2004 by the Food and Agriculture Organization (FAO) to share and implement action plans for managing and controlling serious invasive species in sub–Saharan Africa (Lyimo *et al*., 2009).

Despite having laws, institutions, conventions, and protocols, which prevent the introduction of alien species, new potentially harmful IAPs have often been reported in the country (Lyimo *et al*., 2009). In addition, there is no clear long term strategies dealing with the management of invasive species (Lyimo *et al*., 2009). Thus, there is a need for the ecologists to find viable and sustainable alternative means of managing alien invasions.

1.2 Statement of the Problem

Despite the growing knowledge about *P. hysterophorus* and its harmful impacts on biodiversity, environments and human welfare, information for its current distribution in natural or semi–natural habitats is still lacking in Tanzania. Similarly, management methods to control its invasions are limited in the country. Arusha National Park, which is found in the Arusha region of Tanzania, is more vulnerable to *P. hysterophorus* invasion compared to other protected areas because its immediate surroundings are already invaded with *P. hysterophorus*. Local communities' socio–economic activities (e.g. agriculture, grazing, collection of fodder and fuel wood) nearby the park may influence *P. hysterophorus* seeds dispersal into the border zones of ANP. These border zones are important as they can easily promote the spread of the invasive seeds into the park. *Parthenium hysterophorus* can also enter into ANP as a contaminant of travellers, tourists and staff, in particular as seeds in their belongings (Gervilla *et al*., 2019), or with seeds carried in mud adhered to their shoes into the park. Similarly, vehicles from invaded areas in Arusha may carry *P. hysterophorus* seeds in mud adhered to the tyres into the park and /or its border zones. This study sought to assess the current and potential distribution of *P. hysterophorus* within ANP and areas adjacent to its

border zones. If current *P. hysterophorus* distribution is known and can be predicated, it can enable planning management to direct control efforts to areas with current and potential invasion.

1.3 Rationale of the Study

Current control techniques of *P. hysterophorus* (i.e. biological, chemical, mechanical removal) in some countries have not been effective to suppress the invasive (Adkins & Shabbir, 2014; Brunel *et al*., 2014; Shabbir *et al*., 2013; Shrestha *et al*., 2015). In Tanzania, experimental approaches to control *P. hysterophorus* have been rare and little is known about how the invasive can be controlled. For example, forage plant species have not been tested experimentally for their efficiency to suppress *P. hysterophorus* growth vigour in competition experiments. Although bio–herbicide extracts of native or non–invasive plant species might be a potential management tool for controlling *P. hysterophorus* in Tanzania, very limited studies have investigated their bio–herbicide potential to suppress IAPs.

The evidence of *P. hysterophorus'* negative impacts on crop productivity, biodiversity, and composition of native plants through allelopathy (Ayele *et al*., 2013; Bajwa *et al*., 2019; Miranda *et al*., 2014; Osunkoya *et al*., 2017; Timsina *et al*., 2011) and competition for resources such as space, light, nutrients and water is growing (Shabbir *et al.*, 2013; Shabbir & Bajwa, 2006; Shrestha *et al*., 2015; Tamado & Milberg, 2000). However, there remains a gap in knowledge about its impact on pollination services for co–flowering plants. Thus, this study assessed bio–herbicide potential of *D. uncinatum* leaf crude extract, and suppressive ability of legume fodder plant species *Lablab purpureus, Desmodium intortum* and *Medicago sativa* in controlling *P. hysterophorus* growth. It also investigated the impact of *P. hysterophorus* on soil–chemical properties, and foraging behaviour and visitation of insect flower visitors to co–flowering plant species.

1.4 Objectives

1.4.1 General Objective

The primary objective of the study is to assess the distribution, management and impact of *Parthenium hysterophorus* on insect flower visitation to co–flowering plants and soil chemical properties in Tanzania.

1.4.2 Specific Objectives

(i) To assess current *P. hysterophorus* distribution within and at the border zones of Arusha National Park (ANP).

(ii) To assess whether *P. hysterophorus* affects soil–chemical properties in the invaded locations.

(iii) To investigate if *P. hysterophorus* suppresses pollinator visitation to co–flowering plants.

(iv) To assess whether combinations of forage plant species in multi–species plant communities can outcompete *P. hysterophorus.*

(v) To assess whether *D. uncinatum* leaf crude extract suppresses *P. hysterophorus* growth.

1.5 Research Questions

(i) What is the current *P. hysterophorus* distribution within and at the border zones of ANP?

(ii) Does *P. hysterophorus* affect soil–chemical properties?

(iii) Does *P. hysterophorus* affect pollinator visitation to co–flowering plant species?

(iv) Does *P. hysterophorus* affect pollinators' visitation rate on co–flowering plants?

(v) Does *P. hysterophorus* reduce the number of arriving pollinators on co–flowering plants?

(vi) Does the combination of forage plant species in multi–species competitive communities suppress *P. hysterophorus* growth?

(vii) Does *D. uncinatum* leaf crude extract suppress growth vigour of *P. hysterophorus*?

1.6 Hypotheses

(i) *Parthenium hysterophorus* invasion affects soil–chemical properties.

(ii) *Parthenium hysterophorus* has negative impacts on pollination dynamics in habitats it invades, specifically:

(a) It decreases the pollinator visitation rate on co–flowering plants.

(b) It reduces the number of arriving pollinators on co–flowering plants.

(iii) The combination of forage plant species in multi–species competitive communities suppresses *P. hysterophorus* growth more strongly than just one competitive forage plant species alone.

(iv) *Desmodium uncinatum* leaf crude extract suppresses *P. hysterophorus* growth vigour.

1.7 Significance of the Study

Awareness and knowledge about the distribution of the invasive species are important for the management of biological invasion. This study establishes current spread of *P. hysterophorus* within and around the border zones of ANP. This serves as a baseline for future studies and management of *P. hysterophorus* invasion in protected areas. Knowing the current distribution of *P. hysterophorus* can inform the ANP, Tanzania National Park (TANAPA) and Tanzania Wildlife Research Institute (TAWIRI) to take immediate collective action to control the invasive before it invades other protected areas in the country. In addition, it can help to reduce the cost of managing the invasions by directing the management efforts to areas with potential spread. The study also raises awareness to conservation managers about the presence of *P. hysterophorus* within and surrounding border zones of the ANP and its adjacent villages. Thus, it is vital to carry out a survey in order to document the spread of *P. hysterophorus* in this area (ANP) of high economic significance. The survey information collected can further aid in monitoring of *P. hysterophorus* through repetitive surveys, thereby facilitating tracking of the invasive.

Furthermore, this study is vital to smallholders and extension officers as it discourses the impact of *P. hysterophorus* to pollination services on co–flowering plants, and soil chemical properties. This raises awareness about the urgency of controlling the invasive to avoid competition for flower visitors between *P. hysterophorus* and flowering crops and hence, loss or decrease of crop yield as a result of reduced pollinators. In addition, the study provides an ideal approach of using crude extracts of native or non-invasive plants with bio–herbicide

potential to control *P. hysterophorus*. This should catalyse researchers to conduct further studies to investigate more native plants with bio–herbicide traits able to control *P. hysterophorus* and other invasives in Tanzania.

This study, study also highlights the use of suppressive fodder plant species as an approach of controlling *P. hysterophorus*. These fodder plants can additionally be used as forage for livestock, and for controlling invasive weeds in agroecosystems. Competitive fodder plant species may be planted on private lands, and/or protected areas to maintain species density and /or diversity, and thus, enhance ecosystem resilience against invasions. The study is opportunity for further research on *P. hysterophorus* ecology and management techniques. Moreover, it will be a good reference for invasion biologists, botanists, entomologist, agricultural community, students and researchers.

1.8 Delineation of the Study

This study was carried out to study the impact of *P. hysterophorus* on soil chemical properties and pollinator visitations in the invaded habitats using only five permanent plots. However, it would have been prudent to have larger replication sizes (i.e. more than five plots) for the number of sites. Soil sampling and field surveys were conducted only once during the study. Several sampling and surveys would have been conducted to provide a broad understanding of the impact of *P. hysterophorus* on the soil properties and its distribution within and outside the ANP. Further, the experiments to investigate suppressive effect of *D. uncinatum* leaf crude extract and fodder plant species to control growth vigour of *P. hysterophorus* had short duration. This duration did not cover the whole growth season of focal species. Furthermore, experiment particularly pot experiment was conducted under controlled environments. Thus, it would have been practical to extend the duration of experiments to cover entire growth season of the species so that the related findings may be extrapolated to the real field conditions.

CHAPTER TWO

LITERATURE REVIEW

2.1 Biology of *Parthenium hysterophorus*

Parthenium hysterophorus L. (1753) bears numerous common English and local or vernacular names given in the countries where *P. hysterophorus* has established (CABI, 2019; Kaur *et al*., 2014). These names may reflect a specific trait, utility, or character of *P. hysterophorus* (CABI, 2019). International common English names include but are not limited to carrot grass, congress grass, ragweed parthenium, carrot weed, Santa–Maria, whitetop weed, and whiteheads (Annapurna & Singh, 2003; CABI, 2019; Kohli *et al*., 2006). Nonetheless, the most common used name is Parthenium weed. *Parthenium hysterophorus* is a genus of sixteen shrubs, herbaceous perennials and annual species in North and South America (Brunel *et al*., 2014; Kaur *et al*., 2014; Usharani & Raju, 2018; Wakjira *et al*., 2009). However, only *P. hysterophorus* is invasive in the novel range and tends to become a dominant species and crowd out native plants (Adkins & Shabbir, 2014). It has finely lobed leaves and a deep tap root with forked lateral roots, as well as branched inflorescence bearing creamy–white flower heads or capitula (Brunel *et al*., 2014; Usharani & Raju, 2018). *Parthenium hysterophorus* stem is hairy, erect and branched, which become tough and woody with age (Kaur *et al*., 2014). Its flowers (4 to 10 mm in diameter) arise from the leaf branches and produce about 4 to 5 small black wedge shaped seeds (2 to 3.5 mm long) each (Adkins & Shabbir, 2014; Dhileepan, 2012). Only a few seeds can be shed earlier, but most remain in the capitula until senescence (Kaur *et al*., 2014).

Parthenium hysterophorus flowers develop when the plant is 30 – 45 days old after germination (Adkins & Shabbir, 2014; Usharani & Raju, 2018). It may continue to flower for further 180 – 240 days in suitable temperatures and soil moisture conditions (Adkins & Shabbir, 2014). On average, an individual *P. hysterophorus* plant produces approximately 810 flower heads, 624 million pollen grains, and 10 000 – 25 000 seeds (Kaur *et al*., 2014). In addition, *P. hysterophorus* flowers, roots, seeds and shoot contain allelopathic compounds which makes it toxic to native flora and fauna (Adkins & Shabbir, 2014; Kaur *et al*., 2014; Shabbir *et al*., 2013). The most toxic allelochemical in *P. hysterophorus* responsible for the health hazards to humans and livestock is a bitter glycoside parthenin, a main sesquiterpene lactone (Adkins & Shabbir, 2014; Nyasembe *et al*., 2015; Patel, 2011).

2.2 Life Cycle of *Parthenium hysterophorus*

A typical life cycle of *P. hysterophorus* begins with the dispersal of seeds by agents such as winds, water currents or floods, and as contaminant of soil, harvested materials (crops, fodders etc.) and farm machineries (Kaur *et al*., 2014). The plant reproduces only by seeds, and is incapable of reproducing by apomixis or vegetatively from plant parts (CABI, 2019; Dhileepan, 2012). Its flowers are considered to be both entomophilous and anemophilous, i.e. pollinated either by insects (e.g. bees, butterflies and flies) and wind, respectively (Usharani & Raju, 2018). *Parthenium hysterophorus* seeds can live for 4 to 6 years in the soil seed bank (Kaur *et al*., 2014; Navie *et al*., 2004) and germinate all–year round as long as there is sufficient moisture in the soil (Kaur *et al*., 2014). *Parthenium hysterophorus* takes 28–42 days to grow from seeds, rosette (or juvenile) to mature plant and 112–150 days to complete its life cycle (Kaur *et al*., 2014; Tanveer *et al*., 2015). It can grow up to 2 m in height (Knox *et al*., 2011) and completes 4–5 generations per year under favourable conditions (Tanveer *et al*., 2015). *Parthenium hysterophorus* may only die under extremely harsh conditions such as winter frost, prevalent drought or complete shading which prevents light penetration (Navie *et al*., 2004). In general, its life span and flowering duration is significantly determined by the soil moisture (Navie *et al*., 2004). Plate 2 depicts the flowers, seeds, stem and rosettes of *P. hysterophorus*.

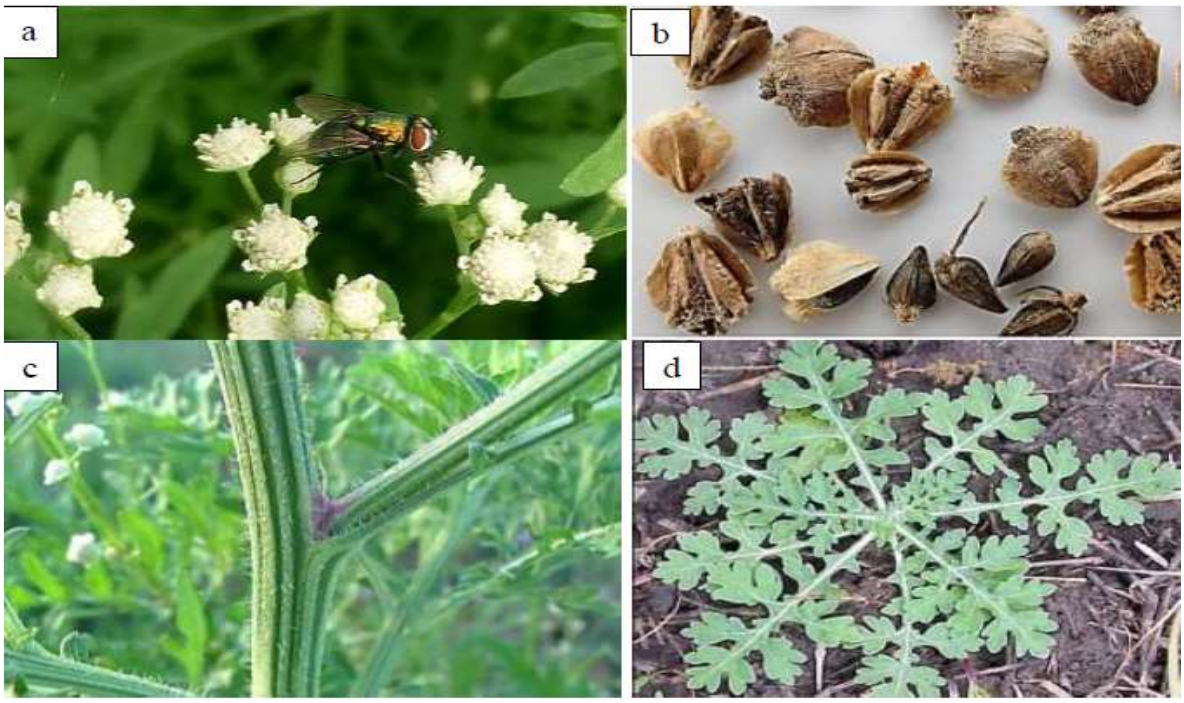


Plate 2: *Parthenium hysterophorus* (a) Flowers, (b) Seeds (ca. 2 mm in size), (c) Stem and (d) Rosette

2.3 Ecology of *Parthenium hysterophorus*

Parthenium hysterophorus grows across a wide range of habitats and soil types such as alkaline, black, clay and cracking soils of high fertility because it can endure severe environmental conditions, i.e., saline, drought and moisture stress (Kaur *et al*., 2014; Upadhyay *et al*., 2013). Nevertheless, its seeds are unable to germinate in soil below a depth of 5 cm (Adkins & Shabbir, 2014). Though its growth rates may differ across soil types, studies found that *P. hysterophorus* grows better on alkaline clay loam soils where annual rainfall is greater than 500 mm (Brunel *et al*., 2014; Tanveer *et al*., 2015; Timsina *et al*., 2011). Annapurna and Singh (2003) reported that *P. hysterophorus* seedlings grown on soils with high clay content enhanced shoots biomass and growth rate in diameter and height, but reduced root growth and prolonged the rosette stage. The average minimum and maximum temperatures for *P. hysterophorus* seed germination is 10 ºC and 25 ºC respectively (Brunel *et al*., 2014; Tamado *et al*., 2002). The optimum soil pH for seed germination is 5.5–7.0, but it can germinate over a wide range of pH (2.5–10) (Kaur *et al*., 2014). When *P. hysterophorus* is freed from controlling agents or natural enemies, e.g. the leaf–eating beetle *Zygogramma bicolorata* (Chrysomelidae), it increases its biomass and suppresses native flora in a recipient ecosystem (Nguyen *et al*., 2017; Shabbir *et al*., 2016).

Parthenium hysterophorus normally invades degraded, disturbed, overgrazed pastures, bare soil, and areas with poor grass cover (Bajwa *et al*., 2019; Nishanthan *et al*., 2013). However, it cannot establish in habitats with intact natural vegetation or pastures. Often it grows along road sides, railway tracks, landfills, around buildings, and in low elevation areas (Etana *et al*., 2015). Thus, changes in land–use patterns due to habitat degradation increase the prevalence of *P. hysterophorus* invasions (Ayele *et al*., 2013; Kaur *et al*., 2014; Nishanthan *et al*., 2013). Its invasions into ecosystems is also promoted by its prolific capacity, large seedbank and rapid seed germination (Dhileepan, 2012; Kaur *et al*., 2014; Nishanthan *et al*., 2013; Roy & Shaik, 2013). Research to investigate physiological mechanisms, which enhance *P. hysterophorus* invasibility in novel locations, are required. This might help to develop methods to interfere with its life cycle and subsequently, control its invasions globally.

2.4 Global Distribution of *Parthenium hysterophorus*

Parthenium hysterophorus is native to North and South America but is an invasive species outside its natural range (Brunel *et al*., 2014; CABI, 2019; Kaur *et al*., 2014). It has invaded

more than 20 countries (CABI, 2019; Safdar *et al*., 2015). Figure 2 depicts global distribution of *P. hysterophorus* as invasive and non–invasive. Like other IAPs, *P. hysterophorus* perhaps found its way to new territory as a contaminant of imported seeds or grain intended for consumption, processing or planting (Axmacher & Sang, 2013; Brunel *et al*., 2014; Gervilla *et al*., 2019; Nigatu *et al*., 2010; Shackleton *et al*., 2017).

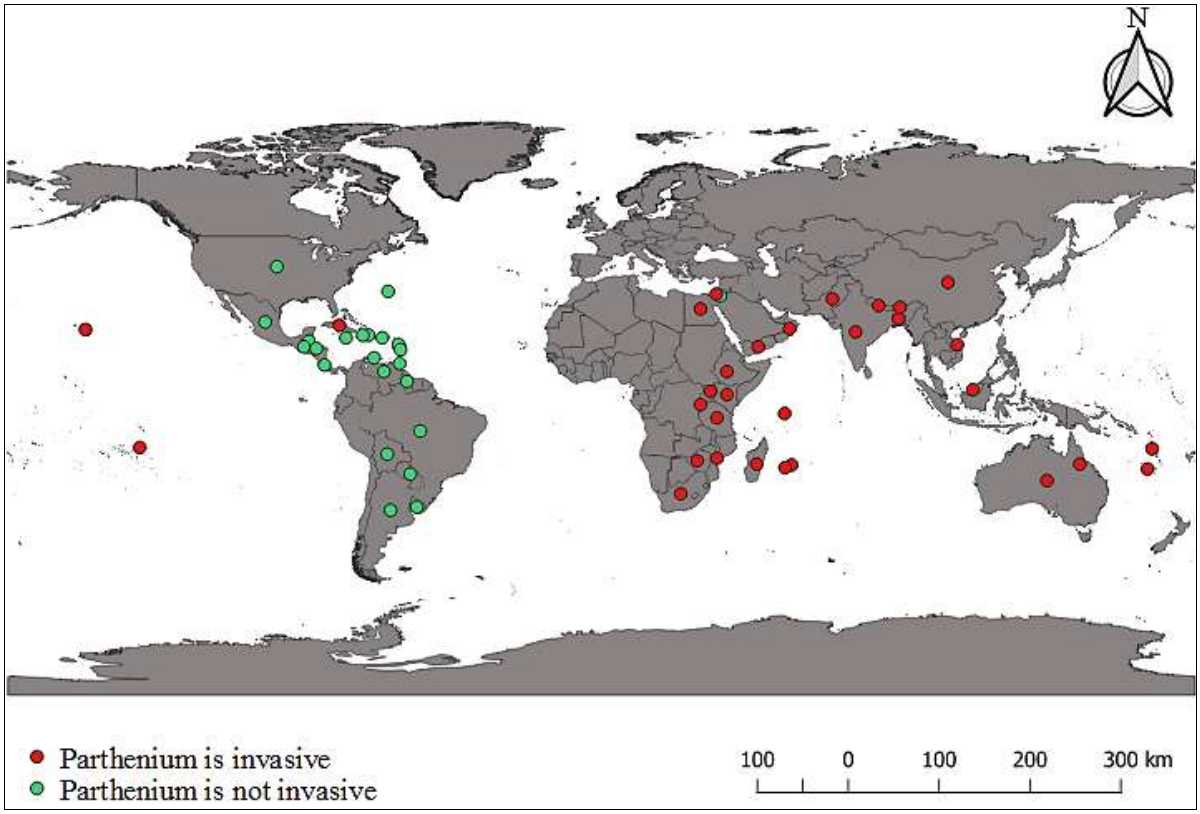


Figure 2: Global Distribution of *Parthenium hysterophorus*. Source of data: CABI (2019). Red Dots Indicate where *P. hysterophorus* has been Introduced and has Become Invasive. Green Dots are where it has been Introduced, but has not yet Become Invasive

In Asia, it is a major invasive in Bangladesh, India, Pakistan, Nepal, Israel, Taiwan, southern China, Sri Lanka, and Vietnam (Bajwa *et al*., 2019; CABI, 2019; Nigatu *et al*., 2010; Shrestha *et al*., 2015). It is also invasive in Australia, New Caledonia, Vanuatu, Papua New Guinea, Hawaii, Christmas Island and New Caledonia (Adkins & Shabbir, 2014; Nguyen *et al*., 2017). In African countries such as Egypt, Eritrea, Ethiopia, Kenya, Mauritius, Madagascar, Mozambique, Somalia, South Africa, Réunion and the Seychelles, Swaziland, Uganda, Zimbabwe and Tanzania (CABI, 2019; Etana *et al*., 2011; Nigatu & Sharma, 2013; Nyasembe *et al*., 2015; Strathie *et al*., 2011; Witt *et al*., 2018), *P. hysterophorus* has colonized rangelands, farmlands, grazing lands, road sides, nature reserves and protected

areas (Ayele *et al*., 2013; Kija *et al*., 2013; Mcconnachie *et al*., 2011; Seta *et al*., 2013; Van der Laan *et al*., 2008; Witt *et al*., 2018). Its invasion is associated with negative impacts on environments (e.g. reducing population size of native plants) and smallholders' livelihoods (Safdar *et al*., 2015, 2016).

Previous studies found that rapid climate change along with elevated atmospheric CO_2 can benefit C_3 species and their biomass production is likely to be greater than that of C_4 plants (Dukes & Mooney, 1999; Moore *et al*., 1987). *Parthenium hysterophorus*, being a C_3 plant species, grew taller at higher CO_2 concentrations (Moore *et al*., 1987; Navie *et al*., 2005; Navie *et al*., 2004). When *P. hysterophorus* was grown under a high CO_2 concentration (550 μmol mol^{-1}), it also produced more branches (35%), greater dry biomass (38%) and more seeds per plants (37%) than when it was grown in ambient CO_2 concentration (380 μmol mol^{-1}) (Shabbir *et al*., 2019). This indicates that *P. hysterophorus* invasion range may increase in the future under climate change and make the management of rangelands and ecosystems comprising merely C_4 species more problematic (Dukes & Mooney, 1999; Moore *et al*., 1987; Navie *et al*., 2005). In this regard, *P. hysterophorus* invasion and proliferation are expected to increase globally.

Having knowledge about the current and potential future distribution of *P. hysterophorus* is imperative for planning effective management of the invasion (Suárez-Mota *et al*., 2016; Taylor *et al*., 2012). Models that predict potential distribution of IAPs are vital tools (Adhikari *et al*., 2015; Thapa *et al*., 2018) because they inform and enable on–ground control of biological invasions (Adams *et al*., 2015; Suárez-Mota *et al*., 2016). Species distribution models (SDMs), for instance, MaxEnt and agent based models (ABM) are scientifically recognised tools for assessing and predicting the distribution of IAPs (Adams *et al*., 2015; de Albuquerque *et al*., 2019; Thapa *et al*., 2018). They are increasingly used to predict the invasive range or spatial patterns of IAPs and prioritize localities for early detection and managing invasion outbreaks (Kija *et al*., 2013). These models construct relationships between environmental parameters and species occurrences, and biophysical in the study site (Adams *et al*., 2015; Adhikari *et al*., 2015; Kija *et al*., 2013; Taylor *et al*., 2012; Thapa *et al*., 2018). This study aims to establish the current *P. hysterophorus* distribution within and outsides the ANP in Tanzania to help in managing the species invasion and proliferation.

2.5 *Parthenium hysterophorus'* Negative Impact on the Environment

Globally, *P. hysterophorus* invasions threaten natural community biodiversity (Brunel *et al*., 2014; Clark & Lotter, 2011; Kija *et al*., 2013; Wabuyele *et al*., 2015), agriculture (Ayele *et al*., 2013; Clark & Lotter, 2011; Pratt *et al*., 2017; Terblanche *et al*., 2016), and the delivery of ecosystem services (Tanveer *et al*., 2015; Terblanche *et al*., 2016). It affects various ecosystems on earth and displaces native plant species (Adkins & Shabbir, 2014). It alters native plants' community structure into *Parthenium*–dominated stands by inhibiting the growth of neighbouring co–existing plants using allelochemicals (Shrestha *et al*., 2015; Timsina *et al*., 2011). Its invasion reduces rangeland production (i.e. fodder quantity) and grazing capacity (Tanveer *et al*., 2015; Terblanche *et al*., 2016), as well as pasture quality where it has established (Clark & Lotter, 2011; Pratt *et al*., 2017; Terblanche *et al*., 2016). Heavy *P. hysterophorus* invasions can reduce natural vegetation seed banks and their ability to regenerate (Navie *et al*., 2004). In Ethiopia, Nigatu *et al.* (2010) found that native flora diversity and above–ground dry biomass in grazing lands declined with increasing *P. hysterophorus* invasion level; and Ayele *et al*. (2013) reported a 62.7% decline of grass cover in the rangelands invaded with *P. hysterophorus*. Similarly, in south east Queensland, Australia, *P. hysterophorus* invasion was reported to reduce significantly pasture community diversity (Nguyen *et al*., 2017).

Parthenium hysterophorus invasion was reported to cause minimal impact on soil physico–chemical properties (Etana *et al*., 2015; Osunkoya *et al*., 2017). A study in central Nepal reported that total soil nitrogen, organic matter content, available phosphorus, potassium and soil pH were highest in *P. hysterophorus* invaded plots than in non–invaded plots (Timsina *et al*., 2011). In central and south east Queensland *P. hysterophorus* was reported to enhance microbial traits (Osunkoya *et al*., 2017). These changes are asserted to alter ecosystem function and trophic levels in the invaded ecosystems (Timsina *et al*., 2011). Owing to the changes it causes in the invaded ecosystems, *P. hysterophorus* acts as an "ecosystem engineer" (Nigatu *et al*., 2010; Perkins *et al*., 2011).

Parthenium hysterophorus has numerous recorded negative impacts on both domestic livestock and human health. Studies assert that its parthenin causes haemorrhage in internal organs due to tissue damage and ultimately death of livestock when significant amounts of *P. hysterophorus* (10 to 50%) are accidentally consumed (Kaur *et al.*, 2014; Nigatu & Sharma, 2013; Patel, 2011; Usharani & Raju, 2018). It also lessens meat and milk quality (Roy &

Shaik, 2013). If people are repeatedly exposed to *P. hysterophorus*, especially flowers or pollen which contain parthenin, they are likely to suffer from respiratory illness, bronchitis, dermatitis, skin allergies, hay fever and asthma (Kaur *et al*., 2014; Terblanche *et al*., 2016; Usharani & Raju, 2018).

Adkins and Shabbir (2014) report that after *P. hysterophorus* had been introduced in the 1950s in Queensland, the plant had invaded about 17 million ha of grazing pastures by 1994. Losses to the livestock industry in pastoral regions of central Queensland was estimated at AU$22 million per year due cost related to *P. hysterophorus* management and pasture loss (Brunel *et al*., 2014; Nigatu *et al*., 2010). Field experiments conducted in Ethiopia showed a 97% decrease of *Sorghum bicolor* grain yield when *P. hysterophorus* was left uncontrolled (Tamado *et al*., 2002). A study in India revealed yield losses of agricultural crops up to 40% owing to *P. hysterophorus* invasions (Brunel *et al*., 2014). Moreover, field experiments conducted in Pakistan demonstrated a linear decrease in maize plant height, grain yield and grain weight per cob with increasing *P. hysterophorus* density (Safdar *et al*., 2015).

In Tanzania, *P. hysterophorus* invasion has been reported to interfere with fodder, livestock and agricultural productivity, as well as biodiversity conservation (Pratt *et al*., 2017; Witt *et al*., 2018). It is estimated that annual maize production losses to smallholders under *P. hysterophorus* expansion range in Tanzania for 5–10 year timescale will be US$5.6 to US$11.2 million (Pratt *et al*., 2017). Centre for Agriculture and Bioscience International (CABI) also estimated that the present value of time invested by smallholder farmers in managing *P. hysterophorus* in Tanzania is US$0.3 million at basic rate (Pratt *et al*., 2017). Since *P. hysterophorus* invasions are expected to increase in the future, with negative impact on the economy of many rural areas (Pratt *et al*., 2017), its management is essential in order to protect smallholders' livelihoods and ecological integrity of natural areas and rangelands.

Furthermore, it has been reported that flowering IAPs affect pollination and reproductive success of native co–flowering flora (Brown *et al*., 2002; Emer *et al*., 2015; Flanagan *et al*., 2009; Morales & Traveset, 2008) because they attract flower visitors away from native plants (Gibson *et al*., 2013; Molina-Montenegro *et al*., 2008; Nielsen *et al*., 2008). Many invasive plants have attractive flowers (Brown *et al*., 2002; Nielsen *et al*., 2008) with greater amounts of pollen and nectar which attract pollinators (Emer *et al*., 2015; Morales & Traveset, 2008). Most invasives have a generalist pollination syndrome and thus, receive diversity of different flower visitors, both generalist and specialist pollinators, compared to natives (Ballantyne *et*

al., 2015; Blüthgen *et al*., 2008; Brown *et al*., 2002; Chittka *et al*., 1999). Thus, generalist invasive flowering plants may have greater impacts on plant community, for instance, pushing out native specialist plant species from the plant–pollinator interaction networks by drawing flower visitors away from the natives (Ballantyne *et al*., 2015; Blüthgen *et al*., 2008; Brown *et al*., 2002). This makes flowering IAPs outcompete native flowering flora due to limited visits (Albano *et al.*, 2009; Larson *et al.*, 2006). Few pollinator visits can lead to loss or less pollen on the stigma of native flowers (Bartomeus *et al*., 2008; Lopezaraiza–Mikel *et al*., 2007). Conspecific pollen loss on heterospecific flowers decreases the volume of pollen conveyed between conspecific flowers (Jakobsson *et al.*, 2008; Molina-Montenegro *et al*., 2008; Morales & Traveset, 2008). Also, heterospecific pollen deposition on conspecific flowers or stigma can cause stigma clogging (Fang & Huang, 2013; Nielsen *et al*., 2008). Stigma clogging and pollen loss reduce female and male fitness, respectively (Fang & Huang, 2013). This may further result in reduced fruits and seeds production in native (specialist) plant species (Nielsen *et al*., 2008; Tiedeken *et al*., 2015).

For *P. hysterophorus*, its pollen was reported to inhibit the fruit and seed set via allelopathy in peppers (*Capsicum annuum*), eggplant (*Solanum melongena*) and tomatoes (*S. lycopersicum*) and grain filling of *Z. mays* (Brunel *et al*., 2014; Kanchan & Chandra, 1980). However, not all IAPs have negative effects on pollination; some have neutral or positive effects (Albano *et al*., 2009; Bartomeus *et al*., 2008; Molina-Montenegro *et al*., 2008; Nielsen *et al*., 2008; Traveset & Richardson, 2014; Ye *et al*., 2014). Invasive alien plants with negative effects on pollination compete for flower visitors with native plant species, and those with positive effects facilitate pollinators visitation to native co–flowering plants (Moragues & Traveset, 2005; Morales & Traveset, 2008). While many studies have investigated the effect of *P. hysterophorus* on native plant community via allelopathy and competition for resources (Nishanthan *et al*., 2013; Seta *et al*., 2013; Tamado & Milberg, 2000; Wakjira *et al*., 2009), little is known whether *P. hysterophorus* can affect pollinator visitation on co–flowering plants. In this view, studies to investigate the adverse impacts of *P. hysterophorus* on pollination services in invaded habitats are very important.

2.6 Management of *Parthenium hysterophorus*

Managing *P. hysterophorus* invasions is crucial in order that biodiversity conservation and ecosystem integrity (Navie *et al*., 2004; Nguyen *et al*., 2017), recovery of native floras (Flory & Clay, 2009), and famers' livelihoods (Bajwa *et al*., 2019; Knox *et al*., 2011) are assured.

Various techniques such as classical biological control, competitive displacement, synthetic herbicides and mechanical or physical control (Ellison & Cock, 2017; Kaur *et al*., 2014; Kumar, 2009; Lenteren *et al*., 2006; O'Donnell & Adkins, 2005; Shabbir *et al*., 2013) have been tried or used to control *P. hysterophorus*. Different studies (Adkins & Shabbir, 2014; Khan *et al*., 2013; Kumar, 2009; Shabbir *et al*., 2019; Shaw *et al*., 2009), the Global Invasive Species Programme (GISP) and CABI (Ellison & Cock, 2017) advise biological control as effective and environmentally benign method for controlling IAPs.

2.6.1 Biological Control

Biological control employs herbivorous invertebrate pests or plant pathogens to control IAPs (Ellison & Cock, 2017; Hinz & Schwarzlaender, 2004; Kumar, 2009; Lenteren *et al*., 2006; Shabbir *et al*., 2016; Shaw *et al*., 2018). They are often natural enemies which may include insects, bacteria, viruses, fungi, and mites (Ellison & Cock, 2017; Shabbir *et al*., 2016). Though biological control does not wholly eradicate alien invasions, it helps to maintain invasive populations lower than they would be in the absence of biological control agents (Kumar, 2009; Shabbir *et al*., 2016). Unlike other countries, Australia and India have widely used insects as biological control agents to suppress *P. hysterophorus* growth (Dhileepan, 2007; Khan *et al*., 2013; Shabbir *et al.*, 2019). A few examples of biological control agents deployed against *P. hysterophorus* include *Epiblema strenuana* (Lepidoptera: Tortricidae), and *Hypothenamus erudistus* (Coleoptera: Curculionidae) used in India (Kumar, 2009; Shabbir *et al*., 2016); *Smicronyx lutulentus* (Coleoptera: Curculionidae), *Contrachelus albocinereus* (Coleoptera: Curculionidae), *Listronotus setosipennis* (Coleoptera: Curculionidae), *Bucculatrix pathenica* (Lepidoptera: Bucculatrigidae) in Australia (Adkins & Shabbir, 2014; Dhileepan, 2007; Shabbir *et al.*, 2019) and *Zygogramma bicolorata* in India, Pakistan, Nepal, South African and Australia (Dhileepan, 2012; Kaur *et al*., 2014; Shabbir *et al*., 2016; Strathie *et al*., 2011).

In Tanzania, only *Z. bicolorata* introduced from South Africa has been released to control *P. hysterophorus* in a few places in Arusha by the Tropical Pesticides Research Institute (TPRI) (Kilewa, 2018). Biological control through microorganisms (bacteria, fungi and viruses) have also been used in India and Australia to suppress *P. hysterophorus* (Adkins & Shabbir, 2014; Kaur *et al*., 2014; Kumar, 2009; Shabbir *et al.*, 2013). The use of alien insects as biological control agent has received great attention globally because they can cause harmful effects in the released ecosystems (Lenteren *et al*., 2006). When they are introduced in new habitats,

they can attack untargeted native species, establish and become difficult to control (Evans, 1997). To avoid such risks, biological control agents for suppressing *P. hysterophorus* must be host specific or specialised organisms (Shabbir *et al*., 2016; Zhao *et al*., 2008). Also, their efficacy at controlling *P. hysterophorus* may be influenced by temperature, rainfall, native predators, native pathogens, land use change, and elevated CO_2 concentration in the introduced range (Shabbir *et al*., 2019). This is because these factors may affect microclimate, behaviour, population growth and establishment of the biological control agents. Previous studies reported that most biocontrol agents such as the leaf–mining moth *Bucculatrix parthenica* (Lepidoptera: Bucculatricidae), stem–boring weevil *Listronotus setosipennis* (Coleoptera: Curculionidae), and the seed–feeding weevil *Smicronyx lutulentus* (Coleoptera: Curculionidae) have limited impact on *P. hysterophorus* as they are unable to reduce the invasive biomass efficiently (Dhileepan, 2007). These observations suggest that novel research approaches are essential to identify effective but ecologically friendly biological agents to control the invasive plant species.

2.6.2 Chemical and Mechanical Control

Chemicals or synthetic herbicides contribute substantially to the control of *P. hysterophorus* (Adkins & Shabbir, 2014; Kaur *et al*., 2014; Shrestha *et al.*, 2015). Synthetic herbicides such as ametryn, atrazine, bromoxynil, chlorimuron ethyl, glyphosate, metribuzin and 2,4-D EE are considered very effective in suppressing *P. hysterophorus* (Kaur *et al*., 2014). However, due to their potentially detrimental effects on the environment and human health they are discouraged for use in natural areas (Flory & Clay, 2009; Ngondya *et al*., 2016b; Yu *et al*., 2018). They may damage or kill ecologically beneficial species such as insect natural enemies, pollinators and decomposers, as well as soil macrobes and microbes which play significant roles in nutrient cycling (Frimpong *et al*., 2018). Most synthetics herbicides leave toxic residues that prevent native plant recruitment by inhibiting seed germination, or lead to negative effects on a particular plant species (Flory & Clay, 2009). They can also alter soil and water physical–chemical properties (Qasem & Foy, 2001; Yu *et al*., 2018). Furthermore, IAPs may also become immune against synthetic herbicides and, thus, difficult to eradicate (Ngondya *et al*., 2016b).

Mechanical or physical control (i.e., uprooting and burning) is also considered an effective method for controlling *P. hysterophorus* invasions (Adkins & Shabbir, 2014; Kaur *et al.*, 2014). Through this technique, invaded ecosystems can be restored, and native plants re–

establish (Adkins & Shabbir, 2014; Kaur *et al*., 2014; Lurgi *et al*., 2016). However, the method provides only a short term solution as many IAPs have large seedbanks (Qasem & Foy, 2001). Hand–weeding is also a time consuming, labour–intensive and costly technique in large areas (Kaur *et al*., 2014; Shabbir *et al*., 2016). While non–target species might be affected through soil disturbance and disruption of roots or mycorrhizal networks during uprooting (Flory & Clay, 2009), people may suffer from health problems as a result of direct skin contact with *P. hysterophorus* (Kaur *et al*., 2014; Seta *et al*., 2013).

If uprooting is conducted after seed setting of *P. hysterophorus* plants, one would expect that it will enhance invasion in the area (Kaur *et al*., 2014). Thus, *P. hysterophorus* should be removed when it is at rosette stage and followed by sowing competitive plants, perennial pasture or crops. Moreover, burning as a strategy is not a viable approach to manage the invasive because it needs large quantities of fuel, but also destroys other valuable and economically important plant species. Methods other than burning may be used for controlling *P. hysterophorus* to avoid promoting the invasions by reducing natural vegetation biomass in the invaded habitat.

2.6.3 Bio–herbicide Approach

Bio–herbicides are natural products derived from living organisms to suppress invasive growth. These include, but are not limited to, plant–derived natural products and microbial (bacteria, fungi, virus, nematodes) metabolites (Christina *et al*., 2015; Kaur *et al*., 2014; Singh *et al*., 2005; Zhao *et al*., 2008). Bio–herbicides are regarded as a form of inundative biological control (Bailey, 2014; Kaur *et al*., 2014). Earlier studies have revealed that extracts of allelopathic plants with bio–herbicide potential can suppress the germination and growth vigour of IAPs (Bhadoria, 2010; Christina *et al*., 2015; Hooper *et al*., 2010; Ming Chen & -Lin Peng, 2018; Ngondya *et al*., 2016b; Zhao *et al*., 2008). Bio–herbicides from native or alien plants have been reported to suppress germination, growth and development of *P. hysterophorus* (Evans, 1997; Kaur *et al*., 2014). Most of these studies were conducted under controlled environments, in screen house (pots), field plots, or laboratory. None of the available studies have ever confirmed that bio–herbicide was successfully deployed in a field situation to control an invasive. Hence, trials to test efficacy of bio–herbicides in the field situation is required.

Bio–herbicide extracts are considered a potential management tool to control *P. hysterophorus* as their non–target impacts are likely to be less severe to the environment compared to synthetic herbicides (Christina *et al*., 2015; Zhao *et al*., 2008). Roots, shoots and leaf extracts of several plant species i.e. *Aegle marmelos, Azadirachta indica, Cassia occidentalis, Cenchrus pennisetiformis, Dicanthium annulatum, Eucalyptus citriodora, Eucalyptus tereticornis* and *Sorghum halepense* were reported to inhibit *P. hysterophorus* germination, respiratory activity, seedling growth, and reduced chlorophyll content (Evans, 1997; Kaur *et al*., 2014; Singh *et al*., 2005; Singh *et al*., 2013). Similarly, metabolites of bacteria, fungi, nematodes, and viruses have been used to control *P. hysterophorus* in some countries such as Australia, Pakistan and India (Adkins & Shabbir, 2014; Kaur *et al.*, 2014; Kumar, 2009; Shabbir *et al.*, 2013). For example, a phytotoxin from a fungi *Phoma herbarum* (Vikrant *et al*., 2006) and *Alternaria alternata* (Saxena & Kumar, 2010) investigated in India demonstrated a potential to control *P. hysterophorus*. This study highlights the potential management of *P. hysterophorus* using bio–herbicides and thus, promotes studies to assess native plants and microbial natural products with potential for managing *P. hysterophorus* invasions.

2.6.4 Competitive Displacement

Control of IAPs through competitive plants embraces the use of native or beneficial alien–non–invasive forage species (Ammondt & Litton, 2012; Ngondya *et al.*, 2016a; Shabbir *et al.*, 2013). Competitive displacement is considered the most economic and feasible way of suppressing alien invasives (Čuda *et al*., 2015; Flory & Clay, 2009). Previous studies have demonstrated that competitive suppressive plants can be used to suppress *P. hysterophorus* growth in the invaded habitats (Adkins & Shabbir, 2014; Khan *et al*., 2013; Shabbir *et al.*, 2013). Australia and India have extensively tested and used suppressive fodder plant species (e.g. *Xanthium strumarium, Sorghum halepense, Clitoria ternatea, Panicum maximum, Sida acuta, Croton bonapladanium, Cenchrus pennisetiformis, Digitaria eriantha, Amaranthus spinosus*) to control *P. hysterophorus* (Adkins & Shabbir, 2014; Khan *et al.*, 2019). Other countries where suppressive plants have been tested to control *P. hysterophorus* are South Africa and Pakistan (Khan *et al.*, 2014; Shabbir *et al*., 2013; Shabbir & Bajwa, 2005; Van der Laan *et al.*, 2008). More competitive plants were reported to suppress growth and biomass of *P. hysterophorus* (Khan *et al.*, 2013; Shabbir *et al*., 2013). Butterfly pea legume (*C. ternatea*), purple pigeon grass (*S. incrassata*), and buffel grass (*Cenchrus ciliaris*) suppressed *P.*

hysterophorus growth by >50% under a simulated grazing experiment (Khan *et al.*, 2019). *Cenchrus ciliaris* which is widely planted all over central Queensland by landholders, competes with and suppresses *P. hysterophorus* (O'Donnell & Adkins, 2005). In Australia, the fodder species *C. ciliaris, C. ternatea, Chloris gayana*, *Dichanthium sericeum*, and *Bothriochloa insculpta* reduced *P. hysterophorus* growth by > 62% (Khan *et al.*, 2014). But, in Tanzania, very limited studies have been conducted to identify and use competitive plants for controlling *P. hysterophorus* invasion.

Due to the challenges facing biological control through insects and microorganisms, and manual removal of invasive plants, areas invaded by *P. hysterophorus* may be assisted to recover following invasive removal with the addition of competitive diverse plant species (Ammondt & Litton, 2012; Shabbir *et al.*, 2013; Tracy *et al.*, 2004). Literature show that an increase of suppressive native plant diversity in grassland could reduce ecosystem invasibility (Ammondt & Litton, 2012; Knops *et al.*, 1999; Shabbir *et al.*, 2013; Tracy *et al.*, 2004). Li *et al*. (2015) reported that the invasive weed *Ipomoea cairica* biomass and stem length were significantly depressed when planted together with competitive native plants *Pueraria lobata* or *Paederia scandens*. Knops *et al*. (1999) and Tracy *et al*. (2004) acknowledged that an increase in native plant diversity in grassland reduced ecosystem invasibility. Hence, competitive displacement method (i.e. using competitive suppressive plants) might be the potential tool to improve efficacy of managing *P. hysterophorus* in natural or semi–natural habitats where burning and/ or chemical herbicides application may not be permitted (Christina *et al*., 2015; Evans, 1997; Khan *et al*., 2019; Ngondya *et al*., 2016b).

The two methods (competitive displacement and native plant–derived bio–herbicide) appear to be promising options for controlling IAPs because they are potentially less harmful to the environment. Hence, they must be explored further. The study aimed to assess the potential use of legume fodder plant species (*L. purpureus*, *D. intortum* and *M. sativa*) to compete with and displace (or suppress) *P. hysterophorus* in field plots and in the screen house; and *D. uncinatum* leaf crude extract to inhibit seed germination and growth vigour of *P. hysterophorus*.

2.7 Benefits of *Parthenium hysterophorus*

Despite its largely negative impacts on the environment, some studies have shown that *P. hysterophorus* might also have some beneficial aspects (Kushwaha & Maurya, 2012;

Nyasembe *et al*., 2015; Patel, 2011). The species contains various chemical constituents such as glucosides, histamine, saponin, and triterpene (Adkins & Shabbir, 2014; Roy & Shaik, 2013). These chemicals are thought to have potential to be used as biocontrol against different pathogens as herbicidal, insecticidal, antibacterial, trypanocidal and nematicidal products (Kushwaha & Maurya, 2012). *Parthenium hysterophorus* is also suspected to have medicinal values such as antifungal, antiamoebic and antimalarial (Kushwaha & Maurya, 2012; Nyasembe *et al*., 2015). Patel (2011) and Roy and Shaik (2013) report that *P. hysterophorus* has traditionally been used as a remedy for allergies, asthma, dizziness, dysentery, diarrhoea, vomiting, malaria, urinary tract infections, psoriasis, nausea, skin inflammation, neuralgia, rheumatic pain and tinnitus in Asia. Although is not tested, there has been an assumption that *P. hysterophorus* can be used as fodder for the livestock when it is detoxified via anaerobic fermentation (Patel, 2011). It can also be used as a source of additives in livestock manure for biogas production, removal of dye and heavy metals from environment, and mitigating other weeds (Patel, 2011). Though *P. hysterophorus* appears to have some benefits, detailed research to investigate the applicability and safety of such uses under different environmental settings is needed.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was conducted in Meru district of Arusha region in Tanzania. A field survey to assess the current distribution of *P. hysterophorus* was conducted within the Arusha National Park (ANP) and neighbouring villages at the border zones (3º 15' S, 37º 00' E). Arusha national park is located between the peaks of Mountain Kilimanjaro and on the eastern side of Mount Meru in Arusha region. The park was established in 1960 (Boshe, 1984) with a total area of 137 km^2. There are two rainy seasons, the long rains (April to May) and the short rains (October to November). The mean annual rainfall for the higher moist areas ranges between 1400 and 2400 mm and lower drier areas 600 and 1300 mm. It is rich in flora (i.e. *Albizia schimperiana Asystasia gangetica, Barleria submollis, Hypoestes aristata, Carissa edulis, Caesalpinia decapetala*, and *Ficus thonningii*) and fauna (i.e. Cape buffalo, Zebra, the Black-and-white colobus monkey, the Blue monkey, Flamingo, Elephant and Bushbuck) which varies with topography (Boshe, 1984).

Field studies were conducted at Tengeru (3° 22.002′ S, 36° 47.008′ E) and Mikuuni–King'ori (3°20.613'S, 36° 59.892' E) to investigate the impact of *P. hysterophorus* on insect flower visitation to co–flowering plants. The mean annual temperature in Tengeru and Mikuuni–King'ori is 19.5°C and 19.6°C, and average annual rainfall is 1078 mm and 1361 mm, respectively. Experiments to investigate suppressive effects of selected competitive fodder plant species and bio–herbicide of *D. uncinatum* leaf crude extract on *P. hysterophorus* growth vigour were carried out at the NM–AIST Tengeru campus (3º 24.149′ S and 36º 47.790′ E).

3.2 Assessing *P. hysterophorus* Distribution Within and Outside Arusha National Park

A field survey was conducted within ANP and villages nearby the park border zones (Fig. 3) between January and June 2018 to collect current distribution data of *P. hysterophorus*. The ANP was surveyed because it is more vulnerable to the invasions as it is located in Arusha region where *P. hysterophorus* invasion is high (Kilewa & Rashid, 2014). Therefore, the probability of *P. hysterophorus* invading ANP is high compared to other protected areas.

Adjacent villages at the border zones were surveyed because human socio-economic activities (farming, livestock keeping and fuelwood collection) may enhance the spread of *P. hysterophorus* and thus, promote its invasion into the park. A survey inside and outside the ANP was conducted along roads using a vehicle and motorcycle respectively. Roads were scanned at both sides for the presence of *P. hysterophorus*. Outside ANP stops were made after every 30 to 50 m to scan *P. hysterophorus* in farms, grazing fields and settlements close to the roads, whereas, inside ANP stops were made at interval of 1 km. The 30 – 50 m interval was chosen in order to ensure sufficient capture of *P. hysterophorus* locations (Thapa *et al*., 2018). Furthermore, whenever *P. hysterophorus* was seen, stops were made and a thorough scanning was conducted in the adjacent areas. The invasive locations were recorded using Garmin etrex20 GPS. Presence data were recorded which comprised latitude, longitude, elevation, land use type and density per square meter. The density of *P. hysterophorus* was visually estimated as high, medium and low when *P. hysterophorus* plants were more than 4, 3–4 and 1–2 individuals in 1 m^2 quadrat respectively.

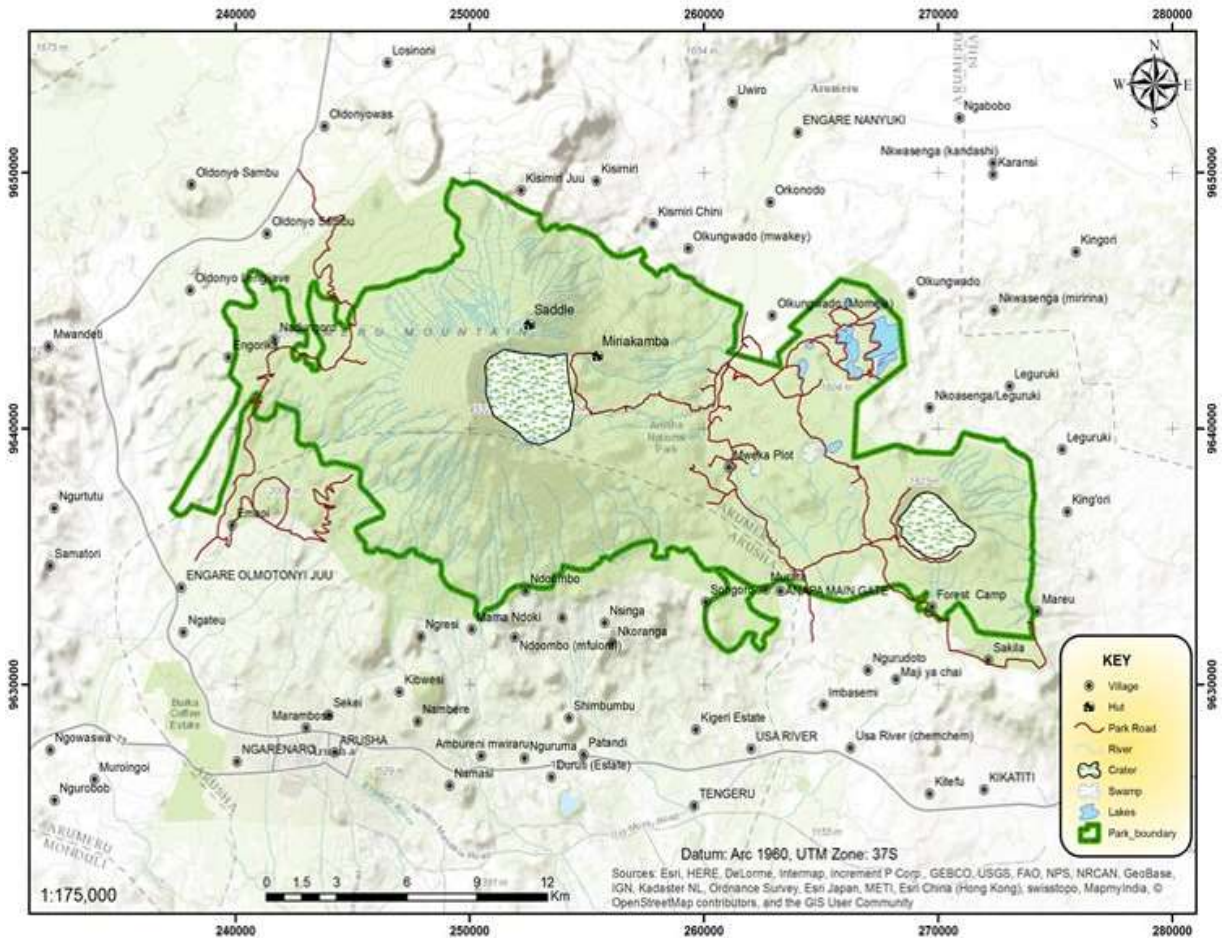


Figure 3: Arusha National Park and Surrounding Villages

The roadside survey method was opted for because *P. hysterophorus* frequently grow along roadsides as it is being spread via seeds on vehicle tyres (Thapa *et al*., 2018; Wabuyele *et al*., 2015). The method also ensures a rapid assessment of the invasive distribution (Christen & Matlack, 2006; Kosaka *et al*., 2010; Von Der Lippe & Kowarik, 2007; Wabuyele *et al*., 2015). Further, the road verges are suitable for colonization by *P. hysterophorus* as they provide suitable microhabitats for the invasive (Johnston & Johnston, 2004; Kosaka *et al*., 2010; Thapa *et al*., 2018). It is also a good method for surveying the population of a single species or early detection of new and known invasive species (Thapa *et al*., 2018). In general, the roadsides are preferential migration corridors of many IAPs, and the starting points for their invasions into adjacent surroundings (Christen & Matlack, 2006; Johnston & Johnston, 2004). The geographical coordinates recorded during our field surveys were used to create a map of *P. hysterophorus* current distribution within ANP and surrounding villages nearby the border zones using a quantum geographic information system (QGIS) version 3.2. *Parthenium hysterophorus* frequency of occurrence was compared at different elevations, land use types and density.

3.3 Assessing *P. hysterophorus* Impact Soil Chemical Properties

During the field survey, patches with and without *P. hysterophorus* invasions (invaded and uninvaded respectively) were randomly selected for soil sampling. One–meter square quadrats were established at each of the selected patches. The invaded and non–invaded patches were 30 m apart. Prior to collection of soil samples, the litter layer was removed and five soil samples, one from the center and one from each of the four corners of the quadrat to a depth of 10 cm were collected using a garden trowel. The five soil samples were pooled to make a single sample for each quadrat. The soil samples were collected from 20 sampling points i.e. 10 from invaded and another 10 from non–invaded patches. The samples were individually placed into zip–lock plastic bags and transported to the laboratory at the Ministry of Agriculture Training Institute (MATI) at Uyole in Mbeya region of Tanzania for analyses.

The soil was sieved through 2 mm fine–mesh screen to get rid of fine rocks, roots, and other unwanted particles. The soil samples were then analysed for chemical properties i.e. organic carbon (OC), pH, Electrical conductivity (EC), Organic matter, Total nitrogen (Total N), available Phosphorus (P), Calcium (Ca), Magnesium (Mg), Potassium (K), Manganese (Mn) and Cation exchange capacity (CEC). Recommended standard soil analytical methods were used (Osunkoya *et al*., 2017; Osunkoya & Perrett, 2011; Perrett *et al*., 2012). Total OC was

determined by the Tinsley method; the pH was measured potentiometrically in a soil–distilled water suspension (ratio 1:2.5); EC with a saturated soil paste; organic matter and total N were determined by the Walkley–black and Kjeldahl methods respectively; 0.5 M $NaHCO_3$ used to extract the available P and analyzed colourimetrically with the ascorbic acid molybdate method according to Bray and Curtz No 1; $NH_4C_2H_3O_2$ extracted soil cations (Ca^{2+}, Mg^{2+} and K^{+}) and analyzed on atomic absorption spectrophotometer with flame atomizer (Perkin-Elmer Analyst 100); diethylene triamine pentaacetic acid (DTPA) was used to extract Mn; and CEC was determined with Ammonium Acetate method at pH 7.0.

3.4 Assessing *P. hysterophorus* Impact on Flower Visitation to co–Flowering Plants

3.4.1 Characteristics of Study Plant Species

In addition to characteristics described earlier in sections 2.1–2.3 above, *P. hysterophorus* contains hundreds small white flower heads per plant which produce abundant pollen and nectar (Kaur *et al*., 2014; Kushwaha & Maurya, 2012). Its inflorescence, which is corymb like, benefits from insect pollination as well as wind (Kushwaha & Maurya, 2012; Usharani & Raju, 2018). Target study plants *Ocimum gratissimum* (Lamiaceae) and *Ageratum conyzoides* (Asteraceae) (Plate 3) were used as indicator species to investigate the mediated impact of *P. hysterophorus* on flower visitation (the number of arriving flower visitors and visitation rate) and foraging behaviour (duration of visits) of flower visitors on adjacent co–flowering plant species.

Ocimum gratissimum is an erect shrub growing up to 3.0 m tall (Nweze & Eze, 2009) and native to East Africa. It has zygomorphic, nectar–rich inflorescences attracting flower visitors. *Ageratum conyzoides* is an annual erect branched herb with 0.5 – 1.0 m height (Kohli *et al*., 2006). The branched inflorescence of *A. conyzoides* carries pale purple coloured flower heads which are arranged in flat–topped clusters. Though *A. conyzoides* is non–native to Tanzania, it was chosen because (a) it has morphologically similar flowers with *P. hysterophorus* and (b) it was abundant co–flowering plant with *P. hysterophorus* in field sites.

Plate 3: Pictures of (a) *P. hysterophorus* on the Invaded site and (b) its Flowers, (c) *O. gratissimum* and (d) Close Up of its Inflorescence and (e) *A. conyzoides*

The field work was conducted at two sites invaded with *P. hysterophorus*, at Tengeru and Mikuuni–King'ori. Each site (ca. 4 ha) was situated within an agricultural landscape and relatively close (<200 m) to settlements. At each study site, two areas ca. 100 m apart, with and without *P. hysterophorus* invasion (invaded and uninvaded quadrats respectively) were selected for studying foraging behaviour, visitation of flowers and visitation networks. The two sites had similar soil type, vegetation type and coverage. Prior to observations, uninvaded and invaded quadrats were assessed to ensure that the target co–flowering plant species were present.

3.4.2 Observation of Foraging Behaviour and Visitation of Flower Visitors

Five permanent quadrats (plots) of 25 m^2 were randomly established over co–flowering patches within invaded and uninvaded sites at Tengeru between January and April 2018 during *P. hysterophorus* flowering periods. Each quadrat was marked using a marker stick positioned at each corner of the quadrat. Prior to observations, the number of floral units of

each target plant species within each 25 m^2 quadrat was counted. The flower visitors were observed within quadrats over two days per week per month. Each quadrat was observed twice per day, once in the morning (08:00 – 12:00) and once in the afternoon (14:00 – 18:00) for 15 minutes in the same order. The observer noted the number of arriving flower visitors and taxonomic groups in the quadrats. The time spent per flower on the target plant species by each visitor was recorded using a stop watch. Photographs and video clips of flower visitors were taken during field work to aid in identification. Every site was observed on the same day in the absence of rainfall and harsh wind that would affect the activity of flower visitors.

Any flower insect visitor that touched the floral parts or reproductive parts of a flower (anthers or stigmas) of *O. gratissimum, A. conyzoides* and *P. hysterophorus* during the 15 minute period was considered as a potential pollinator (Albrecht *et al*., 2016; Molina-Montenegro *et al*., 2008; Stiers *et al*., 2014; Weissman & Schaefer, 2017). However, the term flower visitor instead of pollinator is used in this dissertation as it was not possible to confirm whether every flower visitor was an effective pollinator. Visit in this dissertation refers to landing of an insect visitor on a flower, which may include probing for nectar and/or pollen, which results in contact with the anthers or stigmas. Flower visitors were identified to taxonomic group level, using the categories; Hymenoptera (honey bees, other bees, wasps and ants), Lepidoptera (brown veined white butterflies, acraea butterflies, monarch butterflies and other butterflies), Coleoptera (blister beetles, ladybird beetles, chafer beetles and other beetles), Diptera (hoverflies, and other flies) and Hemiptera. Visitation rate was calculated according to Stiers *et al*. (2014) as the number of flower visitors to the individual target plant divided by the number of open flowers or inflorescences of that plant within the quadrat to avoid the bias of unequal flower numbers between replicates.

3.4.3 Plant–insect Flower Visitor Network

Considering the absence of studies on flower visitor guilds of *P. hysterophorus* and guilds of flower visitors shared with native flowers, we set out to map the interactions of flower visitors and flowering plants available in the study site. Five 36 m^2 quadrats were randomly established in the invaded and uninvaded sites at Mikuuni–King'ori and Tengeru. Twice a week from April to June 2018, quadrats were observed in the same order, in the morning (08:00 – 12:00) and afternoon (14:00 – 17:00) for 15 minutes while recording plant–flower visitor interactions. Since it was difficult to identify all flower visitors on *P. hysterophorus*

and co–flowering plants to species level in the field, insect visitors were identified by eye where possible, some were photographed, and a representative subset were captured using a sweep net. These preserved specimens were then taken to the University of Dar–es–salaam, Department of Zoology, for identification by an entomologist. Plate 4 depicts some examples of flower visitor guilds of *P. hysterophorus*.

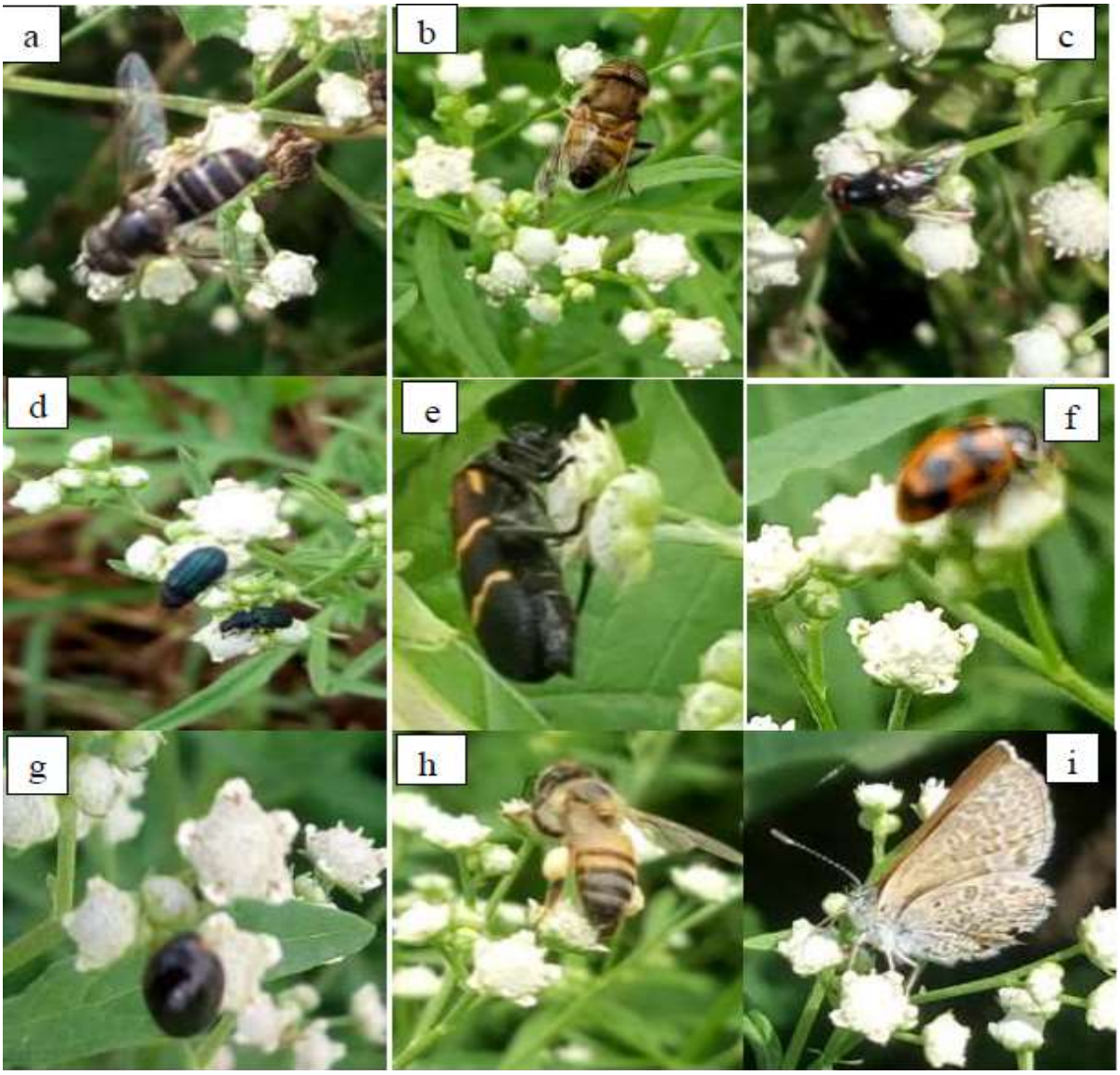


Plate 4: Flower Visitors Foraging on *P. hysterophorus* Flowers: Syrphidae (Hoverflies: (a) and (b), Calliphoridae (c), Melyridae (d), Meloidae (e), Coccinellidae (Ladybird beetles: (f) and (g), Apidae (*A. mellifera*: (h) and Lepidoptera

A quantitative plant–flower insect visitor network or interaction for each site was constructed using R bipartite package 2.08 (Dormann *et al*., 2009) based on the number of visits by flower visitors to each plant species. Interaction networks are tools which help to understand

plant–flower visitor communities and to investigate possible threats to plant diversity and food production if the ecosystem service (pollination) provided by pollinators decreases (Dormann *et al*., 2009). The network–level metrics, such as, network nestedness, connectance, linkage density, number of links per species, network generality and network specialization (H_2') were calculated using the command for network level in the bipartite package (Dormann *et al*., 2009). Nestedness describes the interactions between specialist species and most generalised species (e.g. *P. hysterophorus*) in the network (Adedoja *et al*., 2018). Similarly, it explains the ability of specialists to interact with species that also interact with generalists in the network (Adedoja *et al*., 2018). It confers to interaction network stability where the lower the nestedness value, the lesser stable and vulnerable the network is to disruption (Adedoja *et al*., 2018). Further, the lower nestedness shows a lower randomness level in the species interactions (Blüthgen *et al*., 2008). Nestedness usually ranges from 1 to 100 (Adedoja *et al*., 2018). Connectance is a measure of connections between species or proportion of links observed in a network (Adedoja *et al*., 2018; Blüthgen *et al*., 2008).

Linkage density refers to the mean number of links per species in the network (Adedoja *et al*., 2018; Dormann *et al*., 2009). It takes into account species evenness and richness of the distribution as it describes the distribution degree of interacting species in a network (Ferrero *et al*., 2013; Padrón *et al*., 2009). For large networks, linkage density can be a better network stability descriptor compared to nestedness (Adedoja *et al*., 2018). Generality infers the number of plant flower resources available for species of an insect visitor in the interaction network (Adedoja *et al*., 2018). The higher the generality the more generalised behaviour of species i.e. a flower insect visitor interacting with a larger number of plant species. Network specialization (H_2') estimates the constancy and selection of interaction between species in a network (Adedoja *et al*., 2018). It ranges from 0 (for generalized network) to 1 (for perfectly specialized network) (Adedoja *et al*., 2018; Dormann *et al*., 2009). Thus, presence of *P. hysterophorus* may disrupt these network metrics in plant–pollinator interactions.

3.5 *Parthenium hysterophorus*–Suppressive Plant Species Competition Experiments

3.5.1 Characteristics of Selected Suppressive Plant Species

Selected forage legume plant species *D. intortum* (Fabaceae), *L. purpureus* (Fabaceae) and *M. sativa* (Fabaceae) were investigated for their competitive ability to suppress *P. hysterophorus*

growth (Plate 5). The selected plant species are non–invasive, drought–resistant, fast growing and are used as crop or ground cover (Debela *et al*., 2012).

Plate 5: Test plant species (a) *Lablab purpureus,* (b) *Medicago sativa* and (c) *Desmodium intortum*

Desmodium intortum (Greenleaf Desmodium) and *M. sativa* (Lucerne) have been widely grown in eastern and southern Africa to feed livestock (Aganga & Tshwenyane, 2003; Debela *et al.*, 2012; Ngondya *et al.*, 2016b). *Lablab purpureus* is a crop grown for seed and/or forage production (Amole *et al*., 2013). Its seeds are consumed by some animals including birds and humans (Maass *et al*., 2010). Additional competitive advantage of the test plants over *P. hysterophorus* is their ability to fix atmospheric nitrogen. They are also significant sources of nectar and pollen for honeybees and other flower visitors (Al-Kahtani *et al*., 2017; Bohart, 1958).

Lablab purpureus (Hyacinth bean) is a fast growing herbaceous legume plant in bush form. It can attain a stem height of 3 – 6 m (Aganga & Tshwenyane, 2003; Madzonga & Mogotsi, 2014). It grows in a diverse range of environmental conditions, in bushland, grassland and

forest in the wild because it is highly adaptable and drought resistant (Maass *et al*., 2010). *Lablab purpureus* endures high temperatures range of 18 – 35°C and annual rainfall of 650 – 3000 mm (Madzonga & Mogotsi, 2014). It is a multi–purpose perennial crop grown all over the tropics often as food and forage for human and livestock, respectively (Maass *et al*., 2010). During dry season it remains green and provide fodders to livestock when other forages are scarce and dry (Madzonga & Mogotsi, 2014). Apart from maintaining soil fertility by fixing atmospheric nitrogen, *L. purpureus* can also suppress various invasive weeds (Amole *et al*., 2013). Studies have shown that *L. purpureus* is a good pioneer crop for preparing habitat formerly invaded with alien invasives (Amole *et al*., 2013; Maass *et al*., 2010; Madzonga & Mogotsi, 2014). These traits make *L. purpureus* a good suppressive forage species for controlling *P. hysterophorus*.

Desmodium intortum is another annual tropical forage legume which might suppress *P. hysterophorus*. It grows in areas with annual rainfall of 900 and 3000 mm, and temperatures of 25 and 30°C (Kariuki *et al*., 1999), and its height may range from 1.5 to 7.5 m. This nitrogen fixer also tolerates flooding, waterlogged habitats and shade (Kariuki *et al*., 1999; Maina *et al*., 2006). *Desmodium intortum* takes about 4 months to cover the soil and prevent weeds growth (Maina *et al*., 2006). It is grazed as a long–term pasture, and used as a conservation cover crop because its leaf materials decay mildly in the soil.

Medicago sativa is the third plant identified for this experiment. It is relatively tolerant to drought (Lei *et al*., 2018). It can live for several years within its ideal temperature range of 15 – 25°C, and rainfall of 200 – 2500 mm (Al-Kahtani *et al*., 2017; Lei *et al*., 2018; Radovic *et al*., 2009). It is a widely used legume forage due to its high protein content, high biomass production and adaptability (Al-Kahtani *et al*., 2017). Its erect stem can reach up to 1 m tall with numerous branches (Radovic *et al*., 2009). The *M. sativa* deep root system (4 –7 m) increases its resilience in droughts (Radovic *et al*., 2009). Its seeds can be consumed by humans as food; however, it is often grown and used as a cover crop, hay, silage, and green manure. It is also valued for honey production because its flowers attract honeybees and enhance biodiversity conservation (Al-Kahtani *et al*., 2017; Bohart, 1958). Despite that *M. sativa* lives for 4 – 8 years, it may even survive for more than 20 years depending on climate (Al-Kahtani *et al*., 2017; Latrach *et al*., 2014; Lei *et al*., 2018).

Generally, these test plant species demonstrate three essential characteristics: soil improver, weed competitor, and dense cover crop, which make them possibly appropriate for

management of *P. hysterophorus* in rangelands and protected areas (Kariuki *et al*., 1999; Lei *et al*., 2018; Maina *et al*., 2006).

3.5.2 Competition Experiments

Parthenium hysterophorus seeds were obtained from the Agricultural Division at TPRI in Arusha. *Desmodium intortum* and *M. sativa* seeds were purchased from Kibo Seed Company Ltd. in Arusha. *Lablab purpureus* seeds were obtained from the Department of Sustainable Agriculture, Biodiversity and Ecosystem Management at NM–AIST. Suppressive effects of *D. intortum, M. sativa* and *L. purpureus* on *P. hysterophorus* seedling growth was investigated in field plots (1 m^2) and plastic pots (763.8 cm^2 surface area) at NM–AIST from 10th January to 28th February 2019. Pots were equally filled with black clay soil from uninvaded field plots. Twenty–five seeds of *P. hysterophorus* and test plant species each (*D. intortum, M. sativa and L. purpureus)* were sown in 5 pots and 5 plots at varying combinations in monoculture as a control, and mixtures. Plant seedlings were allowed to grow at a constant density of 4 *P. hysterophorus* / 6 test plants per pot, and 6 *P. hysterophorus* / 10 test plants per plots (Table 2).

Table 2: Pot and Plot Experimental Planting Design Diagram with *P. hysterophorus* and Suppressive Plant Species. Respectively P, M, D and L Stand for *P. hysterophorus, M. sativa, D. intortum* and *L. purpureus.* S_0, S_1, S_2 and S_3 Refer to Levels of Suppressive Species Richness Respectively

***Parthenium hysterophorus* grown with and without suppressive plant species**				**Suppressive plant species grown alone**
PS_0	PS_1	PS_2	PS_3	
Pot experiment				
4P	4P/6M	4P/6M/6D	4P/6M/6D/6L	6M
	4P/6D	4P/6M/6L		6L
	4P/6L	4P/6L/6D		6D
Plot experiment				
6P	6P/10M	6P/10M/10D	6P/10M/10D/10L	10M
	6P/10D	6P/10M/10L		10L
	6P/10L	6P/10L/10D		10D

The total of 11 planting combinations was replicated five times to make 55 planting plots and 55 pots (Table 2). Pots were kept in a naturally illuminated screen–house at NM–AIST. Each

pot and plot was irrigated daily in the morning with 0.5 l and 4 l of water respectively. Positions of pots were randomised twice per week to ensure uniform distribution of sunlight.

Fifty–day–old seedlings of *P. hysterophorus* were harvested from each pot and plot without destroying the roots to assess suppressive effects of test plants on invasive growth. This was assessed by measuring *P. hysterophorus* growth parameters such as stem height, shoot diameter, root length, above– and belowground fresh biomass (AFB and BFB respectively), above– and belowground dry biomass (ADB and BDB respectively) and total fresh biomass. Total leaf chlorophyll content (total Chl) was also determined. Above– and below ground biomass components were measured as an index of plant productivity (Ammondt & Litton, 2012). Seedlings were washed in water to remove dirt prior to separate them into below–and aboveground biomass components. Each component in separate paper bags was dried in an oven at 70ºC for 12 h. Stem height (from soil level to the tip of tallest plant part) and root length were measured using a meter ruler. The shoot diameter (above the first two seedling leaves) and biomass were measured using a digital callipers and an analytical balance respectively.

Competition intensity indices i.e. relative competition intensity (RCI, *eqn. 1*) and relative interaction intensity (RII, *eqn. 2*) were determined to assess performance of *P. hysterophorus* seedlings grown with suppressive species at different diversity levels (Armas *et al*., 2004; Grace, 1995; Weigelt & Jolliffe, 2003). If RCI = 0 there is no competition (neutral), RCI < 0 indicates that the performance of *P. hysterophorus* is better with the presence of suppressive plants (facilitation) and RCI > 0 indicates that suppressive plants have a negative effect on *P. hysterophorus* or competition in the general sense (Armas *et al*., 2004; Vilà *et al*., 2004; Weigelt & Jolliffe, 2003). If RII < 0 competition prevails, RII > 0 facilitation prevails and RII = 0 the outcome is neutral or no interaction. The range of two indices is $1 \geq RCI \geq \infty$- and $1 \geq RII \geq 1^{-}$ (Armas *et al*., 2004; Grace, 1995).

$$RCI = \frac{B_0 - B_w}{B_0} \quad (\text{Grace}, 1995, \text{Armas } et\ al. 2004) \qquad (eqn. 1)$$

$$RII = \frac{B_w - B_0}{B_w + B_0} \quad (\text{Armas } et\ al. 2004) \qquad (eqn. 2)$$

Where: B_0 and B_w are *P. hysterophorus* biomass grown in monoculture and in mixture, respectively. The biomass averages of overall replications of each planting combination were used.

3.6 *Desmodium uncinatum* Leaf (DuL) Extract against *P. hysterophorus* Seed Germination and Seedling Growth

3.6.1 Preparation of DuL Leaf Crude Extract

Desmodium uncinatum fresh leaves were collected from five villages (Nkwaranga, Ngiresi, Sokoni one, Sura and Urisho) in Meru district between June and August, 2018. During this time period the plants were abundant, and weather conditions were suitable (i.e. little rainfall) for collecting leaf samples. The leaves were collected early in the morning before sunrise to avoid possible degradation of any non–photostable allelochemicals. About 10 to 20 leaves were collected randomly from different individual plants occurring on non–agriculture areas, and free from pesticide contamination (Isman & Grieneisen, 2014). Voucher specimens were taken to TPRI for identification.

The leaves were air dried for 30 days under room temperature in indoors to avoid ultraviolet (UV) light to degrade some compounds. Dried leaves were ground into fine powder and stored in porous paper envelopes. Preparation of *D. uncinatum* leaf (DuL) crude extract concentrations followed procedures described by Ngondya *et al*. (2016a), whereby 100 g powder was soaked in 1 l of distilled water to form crude extract. Crude extract was stored in a 4 l plastic container for 72 h in a dark room. The extract was filtered using Muslin cloth and filtrates were diluted with distilled water to obtain different aqueous concentrations of DuL (100 ml each) termed 0 %, 25 %, 50 %, 75 %, and 100 % relative to the original extract.

3.6.2 *Parthenium hysterophorus* Seed Germination Experiments under Dul Extract Treatment

To investigate the allelopathic effect of DuL crude extract on *P. hysterophorus* seed germination, experiments were conducted at NM–AIST, in the lab (for petri dishes) and field (for pots and plots). Five glass petri dishes (each of 70.8 cm^2 surface area), five plastic pots (763.8 cm^2 surface area), and five plots (1 m^2) per treatment were used, and then replicated five times. Petri dishes lined with absorbent cotton wool were rinsed with distilled water before 25 seeds of *P. hysterophorus* were sown in each dish. The same number of seeds were sown in pots and plots. The seeds were kept moist (irrigated ad libitum) with five different

DuL crude extract concentration treatments (0 %, 25 %, 50 %, 75 % and 100 %). Plots were equally spaced 0.5 m apart. The position of petri dishes and pots was randomised weekly throughout the experiment in order to ensure equal distribution of sunlight and more consistent coverage of water. The number of seeds that germinated were recorded daily for 20 days, and the percentage of seeds germinated was calculated. Germination inhibition percentage (IP) of treatments over the control germination were also calculated (*eqn. 3*).

$$\text{IP} = \left(\frac{\text{Germinated seeds in extracts} - \text{Germinated seeds in control}}{\text{Germinated seeds in control}}\right) * 100 \ldots (eqn. 3)$$

3.6.3 *Parthenium hysterophorus* Seedling Growth Experiments under Dul Extract Treatment

Twenty–five field plots of 1 m^2 were planted with 40 seeds each. At the same time, the same number of seeds was planted in each 25 pots using soil from the uninvaded field plots. Plots and pots were watered thoroughly at the time of sowing (0.5 l and 4 l per pot and plot, respectively). Following a week of germination, plots and pots were irrigated twice per week. Seedlings were thinned to three per pot to prevent overcrowding. Twenty–day–old seedlings in plots and pots were sprayed ad libitum using a hand sprayer with five different concentrations of DuL crude extract (0 %, 25 %, 50 %, 75 %, and 100 %) daily for 25 days (2 August to 19 September, 2018). The allelopathic effects of DuL crude extract on seedling growth at different concentrations were investigated by measuring *P. hysterophorus* growth parameters. At the end of the experiments, ten *P. hysterophorus* seedlings per treatment were randomly harvested from each field plot and three from each pot without destroying the roots. Growth parameters i.e. stem height, stem diameter, root length, total Chl, AFB, ADB, BFB and BDB were measured using similar procedures and methods described in section 3.2.2 above.

3.6.4 *Parthenium hysterophorus* Leaf Chlorophyll Content under Dul Extract Treatment and Suppressive Plants Experiments

Five young fresh leaves of 50–day–old *P. hysterophorus* seedlings from competition experiments were selected randomly per field plot and pot to determine total Chl. Also, the same number of leaves were randomly selected from ten *P. hysterophorus* seedlings per field plot and three seedlings per pot sprayed with five different DuL crude extract concentrations. In both experiments, total Chl contents of *P. hysterophorus* seedlings was extracted and

measured as an index of plant health in response to suppressive effects of the competitive forage plants or DuL crude extract treatments. The leaf chlorophyll was extracted according to Hiscox and Israelstam (1979) and Ngondya *et al*. (2016b) with some modification. About 70 mg of *P. hysterophorus* leaves was immersed in 6 ml of dimethyl sulfoxide (DMSO) in a test–tube and incubated at 65°C for 12 h. Afterwards, the extract was made up to a total volume of 10 ml with DMSO, thereafter, transferred to vials for storage (0–4ºC) waiting for analysis.

Three millilitres (3 ml) of *P. hysterophorus* leaf chlorophyll extract was transferred into a microplate to determine absorbance or optical density (OD) of the samples. The OD of the blank liquid (DMSO) and samples was determined under Synergy HTX Multi–Mode Microplate Reader at 663 nm and 645 nm (Hiscox & Israelstam, 1979). Prior to calculating the total Chl, the OD of the blank was deducted from the OD readings of every sample. The equation (*eqn. 4*) was used to calculate the total Chl contents (Hiscox & Israelstam, 1979; Ngondya *et al*., 2016b) respectively, A_{663} and A_{645} are absorbance readings at 663 nm and 645 nm.

$$Total\ Chl = 0.0202A_{663} + 0.00802A_{645} \qquad (eqn.4)$$

3.7 Statistical Data Analysis

Parthenium hysterophorus seedling stem height, shoot diameter, root length, total fresh biomass, above–ground fresh biomass (AFB), above–ground dry biomass (ADB), below–ground fresh biomass (BFB), below–ground dry biomass (BDB) and total Chl content were compared across suppressive species planting mixtures using one–way ANOVA with the number of pots or plots per treatment as the unit of replication. Relationship between *P. hysterophorus* Chl content and total fresh biomass was performed using a Pearson's product–moment correlation analysis. A one–way ANOVA was also carried out to test for differences in *P. hysterophorus* seedlings' growth parameters in various concentrations of DuL crude extract.

The impact of *P. hysterophorus* on visitation (the number of arriving flower visitors and visitation rate) and foraging behaviour (duration of visits) of insect flower visitors to target plants *O. gratissimum* and *A. conyzoides* was analysed using one–way ANOVA (general linear model procedure) with the number of quadrats as the unit of replication and invasion status as categorical predictor. Flower visitor taxonomic groups were compared between the

invaded and uninvaded quadrats. Soil chemical properties were compared between invaded and uninvaded quadrats using t–test.

Prior to data analysis, normality and homogeneity of variance were verified using a Shapiro–Wilk test and Levene's test respectively. Whenever parametric assumptions were not confirmed after transformations using box–cox or log transformation, the non–parametric Kruskal–Wallis test was used. Hemiptera were not compared between sites because their sample size was very small and therefore, they were considered as minor flower insect visitors. The post–hoc Tukey–Kramer HSD (honest significant difference) test was used to compare the significant differences across different planting combinations, as well as foraging and visitation among flower visitors. The results of Kruskal–Wallis i.e. the significant differences in flower visitation and foraging behaviour between flower visitors was separated using a Mann–Whitney Pairwise comparison test. Moreover, the Fisher LSD (least square difference) was used to separate the mean difference across different DuL crude extract concentrations. The statistical software used for all tests was Origin (2013) version 9.0 SR1 at a significance level of 5%, while R version 3.5.1 (2018) was used to construct pollinator visitation network and calculate network level metrics.

CHAPTER FOUR

RESULTS AND DICUSSION

4.1 Results

4.1.1 Distribution of *P. hysterophorus* Within and Outside Arusha National Park

The field survey indicated that *P. hysterophorus* has not yet invaded the ANP. However, the invasion was recorded in some villages (i.e. King'ori, Maleu, Napoco, Ngongongare, Ngurdoto, Oligilai and Sakila) neighbouring the park (Fig. 4). The closest invaded areas to ANP were found in King'ori and nearby Ngurdoto forest reserve (ca. 0.7 km and 0.6 km to the border zone respectively), around Meru view hotel and Migombani (ca.1.0 km and 2.7 km from Ngongongare gate of ANP respectively) and Maleu (ca.1.0 km close to the forest reserve bordering the ANP). Invasion point recorded in Napoco was approximately 3.3 km from Ngongongare gate. Respectively, invaded areas in Sakila, Napoco and Ngurdoto were around 2.5 km, 3.1 km and 3.6 km from Arusha–Moshi road which is highly invaded by *P. hysterophorus*. These distances were estimated in Earth google map.

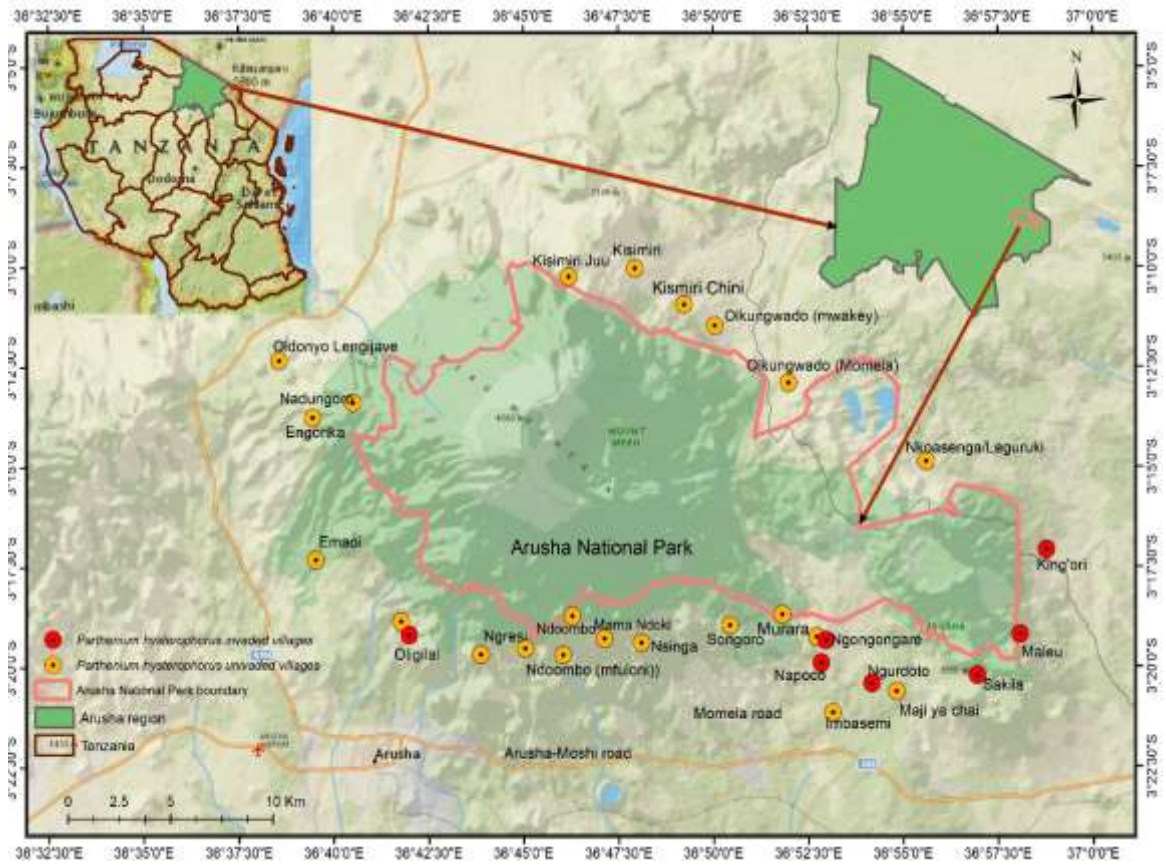


Figure 4: Current spread of *P. hysterophorus* outside the Arusha National Park

Parthenium hysterophorus was observed growing in grazing areas, maize and banana fields. Also, it was observed growing along the Momela road, which enters the ANP through the Ngongongare gate. High density of *P. hysterophorus* was recorded in maize fields, along roadsides, and at lower elevations (Fig. 5). Furthermore, it was observed growing in landfills or dumping ground found near settlements in villages and roadsides.

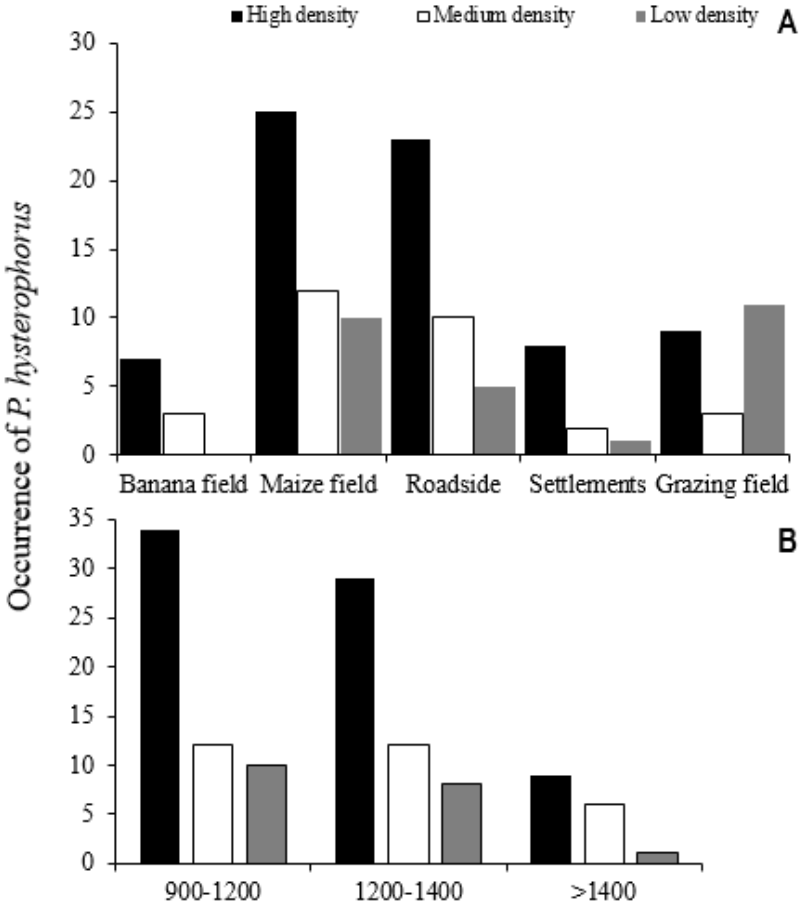


Figure 5: Frequency of *P. hysterophorus* Occurrence in Different Land use Types (A) and Elevation in m.a.s.l (B)

4.1.2 Impact of *P. hysterophorus* on Soil Chemical Properties

Most of the soil chemical properties analysed in the study did not differ significantly between the invaded and uninvaded quadrats ($p > 0.05$, Table 3). A significant difference was observed between the invaded and uninvaded quadrats for pH ($p = 0.013$), P ($p = 0.003$), EC ($p = 0.046$) and Ca ($p = 0.043$) (Table 3). Phosphorus was higher and CEC was lower in uninvaded quadrats compared to invaded ones ($p = 0.003$, $p = 0.015$; Table 3). In this study, it was found that invaded site has high CEC, more acidic soil with lower EC, less calcium and phosphorus.

Table 3: T-test Statistics of the Mean (± SE) Soil Properties in Areas with (invaded) and without (uninvaded) Invasion of *P. hysterophorus*

Soil properties	Uninvaded	Invaded	T	df	*p-value*
Ph	6.54 ± 0.06	6.36 ± 0.03	2.74	18	**0.013**
EC (mS/cm)	0.58 ± 0.08	0.44 ± 0.03	2.14	18	**0.046**
OC (%)	1.79 ± 0.15	1.43 ± 0.22	1.59	18	0.129
Organic Matter (%)	3.09 ± 0.25	2.47 ± 0.38	1.60	18	0.128
Total N (%)	0.11 ± 0.01	0.12 ± 0.02	-0.65	18	0.526
P (mg/kg)	68.62 ± 4.65	48.07 ± 3.69	3.46	18	**0.003**
Ca (Cmol/kg)	19.37 ± 0.35	18.11 ± 0.46	2.18	18	**0.043**
Mg (Cmol/kg)	2.22 ± 0.01	2.21 ± 0.11	0.44	18	0.668
K (Cmol/kg)	3.68 ± 0.26	2.79 ± 0.37	1.98	18	0.063
Mn (ppm)	49.8 ± 7.01	64 ± 3.23	-1.65	18	0.116
CEC (Cmol/kg)	34.4 ± 1.64	43.2 ± 1.83	-2.69	18	**0.015**

Bold *p*-values are significant at $p < 0.05$

4.1.3 Impact of *P. hysterophorus* on Flower Visitation to Co–flowering Plants

The flower visitors on *P. hysterophorus, O. gratissimum*, and *A. conyzoides* comprised a diversity of insect species (Table 4). The number of visits was twice to flowers of *O. gratissimum* and *A. conyzoides* in the uninvaded quadrats compared to invaded quadrats (Table 5). In the invaded quadrats, *P. hysterophorus* received 1209 visits also about twice as many visits compared to the other two indicator plant species (Table 5). Hymenoptera and Diptera were the dominant taxa recorded with greater than 50% of all recorded visits (Table 5). *Apis mellifera* was the most frequent visitor to target species in both invaded and uninvaded quadrats as well as to flowers of both *O. gratissimum* (55%) and *A. conyzoides* (51%) in the uninvaded quadrats (Table 5). *Apis mellifera* also made about 54% of visits to flowers of *P. hysterophorus* compared to *O. gratissimum* and *A. conyzoides* in the invaded quadrats (Table 5). Note that the data of flower visitors are summed over the study period.

Table 4: Flower Visitor Guild of *P. hysterophorus*, *O. gratissimum* and *A. conyzoides*

Order	Common name	Species name or family
Hymenoptera	Honey bees	*Apis mellifera*
	Wasps	Vespidae
	Ants	Formicidae
	Other bees	Xylocopa
Coleoptera	Lunate blister beetles	*Hycleus lugens*
	Black blister beetles	*Epicauta* spp.
	Blister beetles	Meloidae
	Chafer beetles	*Rhabdotis* spp.
	Chafer beetles	*Pchnoda* spp
	Groove-winged flower beetles	Melyris spp.
	Lunate ladybird beetles	*Cheilomenes* spp.
	Potato ladybird beetles	*Epilachna* spp.
	Ladybird beetles	Coccinellidae
Lepidoptera	Brown-veined white	*Balenois aurota*
	African monarch	*Danaus chrysippus*
	Tiny acraea	*Acraea uvui*
	Dancing acraea	*Acraea eponina*
	Encedon acraea	*Acraea encedon*
	Butterflies	*Vanessa virginiensis*
	Flower moths	Scythrididae
	Orange tiger moths	*Secusio* spp
	Chief butterflies	*Amauris* spp
Diptera	Hoverflies	Syrphidae
	Soldier flies	Stratiomyiidae
	Bee flies	Bombyliidae
	Flies	*Stomorhina lunata*
	Flies	Calliphoridae
	Flies	Muscidae
	Drone flies	*Eristalis* spp
	Blowflies	*Chrysomya* spp
	Green bottles	Calliphoridae
	Fruit flies	*Didacus* spp
	Housefly	*Musca domestica L.*

Table 5: Flower Visitors, Number of Insects Observed (N) and Percentage of Frequency (%) of Total Visits that each Pollinator Made to *P. hysterophorus, O. gratissimum* and *A. conyzoides*

Flower visitors	Order	Invaded site						Uninvaded site			
		P. hysterophorus		*O. gratissimum*		*A. conyzoides*		*O. gratissimum*		*A. conyzoides*	
		N	%	N	%	N	%	N	%	N	%
A. mellifera	Hymenoptera	652	54	325	50	226	38	649	55	525	51
Other bees	Hymenoptera	82	7	76	12	108	18	112	10	142	14
Wasps	Hymenoptera	38	3	22	3	34	6	43	4	63	6
Ants	Hymenoptera	0	0	17	3	25	4	8	1	8	1
B. aurota	Lepidoptera	18	1	20	3	9	2	24	2	6	1
Acraea butterflies	Lepidoptera	5	0	7	1	5	1	1	0	5	0
Monarch butterflies	Lepidoptera	13	1	5	1	7	1	9	1	26	3
Other butterflies	Lepidoptera	24	2	18	3	23	4	32	3	23	2
Blister beetles	Coleoptera	52	4	28	4	32	5	43	4	44	4
Ladybird beetles	Coleoptera	57	5	19	3	6	1	42	4	27	3
Other beetles	Coleoptera	23	2	7	1	5	1	24	2	2	0
Hoverflies	Diptera	130	11	47	7	19	3	86	7	74	7
Other flies	Diptera	99	8	48	7	72	12	72	6	43	4
Bugs	Hemiptera	16	1	17	3	17	3	28	2	40	4
Total visits		1209	100	656	100	588	100	1173	100	1028	100

The relative proportion of visits to *O. gratissimum* and *A. conyzoides* by different visitor taxa changed significantly in the presence of *P. hysterophorus*. Both target plants experienced a significant reduction in visits by Hymenoptera (*A. mellifera*, ants, and wasps), most beetles, butterflies, and flies on the invaded quadrats (Table 6). *Post hoc* tests revealed that the number of visits of *A. mellifera* to target plants was significantly higher on the uninvaded quadrats, about twice the number of visits on the invaded quadrats (*O. gratissimum*: $p = 0.0122$; *A. conyzoides*: $p < 0.0001$, Fig. 6). The number of arriving blister beetles ($p = 0.0117$), and ladybird beetles ($p = 0.0157$) to *O. gratissimum*, and ladybird beetles to *A. conyzoides* ($p = 0.0013$) on the uninvaded quadrats was twice as high compared to that on the invaded quadrats (Fig. 7).

Similarly, the number of acraea butterflies visiting *O. gratissimum* ($p = 0.0026$) and other butterflies visiting *A. conyzoides* ($p = 0.0283$) on the uninvaded quadrats was about twice the number of visits on the invaded quadrats (Fig. 8). Moreover, the number of visits of hoverflies ($p = 0.0001$), and other flies ($p = 0.0001$) to flowers of *O. gratissimum* and other flies ($p = 0.0013$) to *A. conyzoides* on the uninvaded quadrats was about three times the number of visits on the invaded quadrats (Fig. 9). Furthermore, the number of arriving chafer beetles, monarch butterflies, ants, other bees and wasps were not negatively affected by *P. hysterophorus*.

Table 6: Kruskal-Wallis and One-Way ANOVA Test Statistics of the Number of Arriving Flower Visitors, Duration of Visits and Visitation Rate of Flower Visitor Functional Groups to Flowers of *O. gratissimum* and *A. conyzoides* on the Invaded and Uninvaded Quadrats

Flower visitor groups	*Ocimum gratissimum*			*Ageratum conyzoides*		
	Number of visits	**Visitation rate**	**Duration of visits**	**Number of visits**	**Visitation rate**	**Duration of visits**
Ants, bees, and wasps	$F_{(7, 32)} = 155.75$*	$H_{(7, 32)} = 35.53$*	$H_{(7, 32)} = 34.98$*	$F_{(7, 32)} = 204.03$*	$H_{(7, 32)} = 34.23$*	$H_{(7, 32)} = 33.22$*
Beetles	$F_{(7, 32)} = 29.79$*	$H_{(7, 32)} = 18$*	$H_{(7, 32)} = 37.52$*	$F_{(7, 32)} = 19.56$*	$H_{(7, 32)} = 31.70$*	$H_{(7, 32)} = 34.14$*
Butterflies	$F_{(7, 32)} = 4.98$*	$H_{(7, 32)} = 11.89$*	$H_{(7, 32)} = 30.35$*	$F_{(7, 32)} = 6.74$*	$H_{(7, 32)} = 16.13$	$H_{(7, 32)} = 28.40$*
Flies	$F_{(3, 16)} = 34.28$*	$H_{(3, 16)} = 14.79$*	$H_{(3, 16)} = 15.61$*	$F_{(3, 16)} = 31.00$*	$H_{(3, 16)} = 6.91$	$H_{(3, 16)} = 16.14$*

Values differ significantly at $p < 0.05$, * indicates significant difference

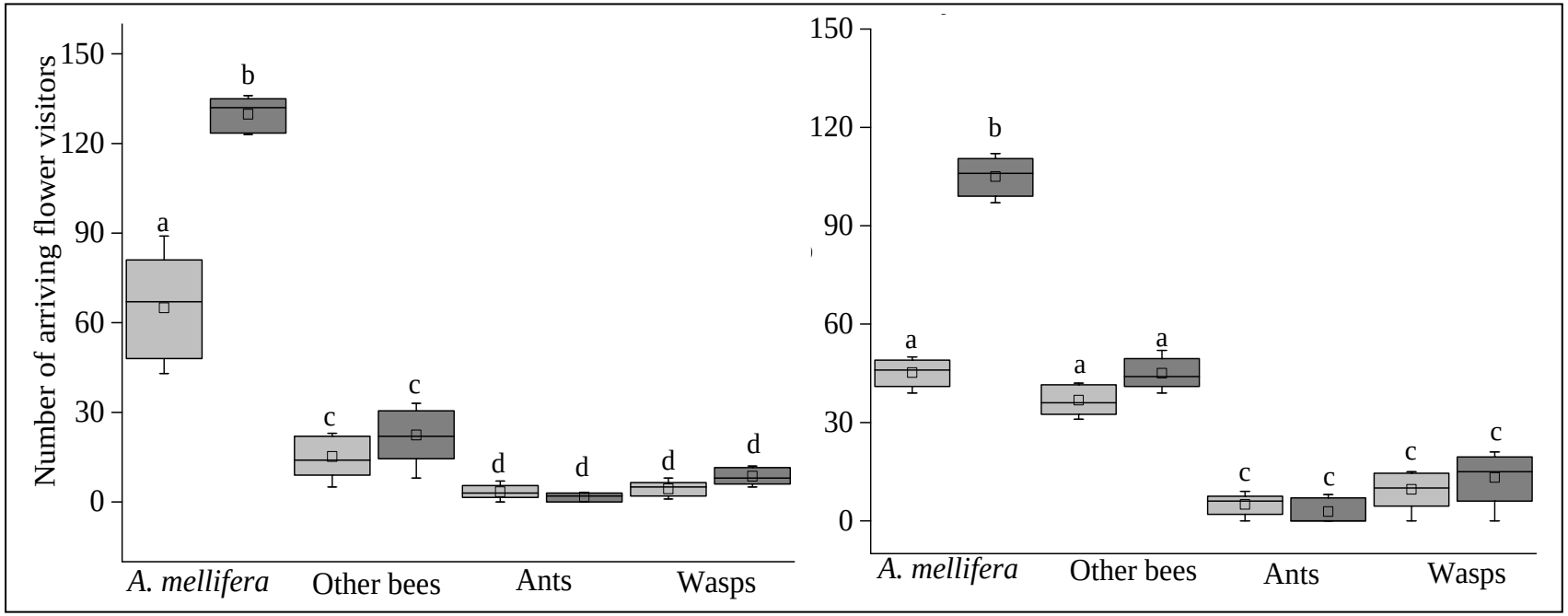


Figure 6: The number of Arriving Hymenoptera to Flowers of *O. gratissimum* (left) and *A. conyzoides* (right) on the Invaded (light grey) and Uninvaded (grey) Sites per 15-min Observation Period. Different Letters on Bars Indicate Significant Differences by Tukey's HSD test at $p = 0.05$. Boxplots show the Mean (Square within Boxes), 25% and 75% Quartile Ranges and Whiskers show the 5th and 95th Percentiles

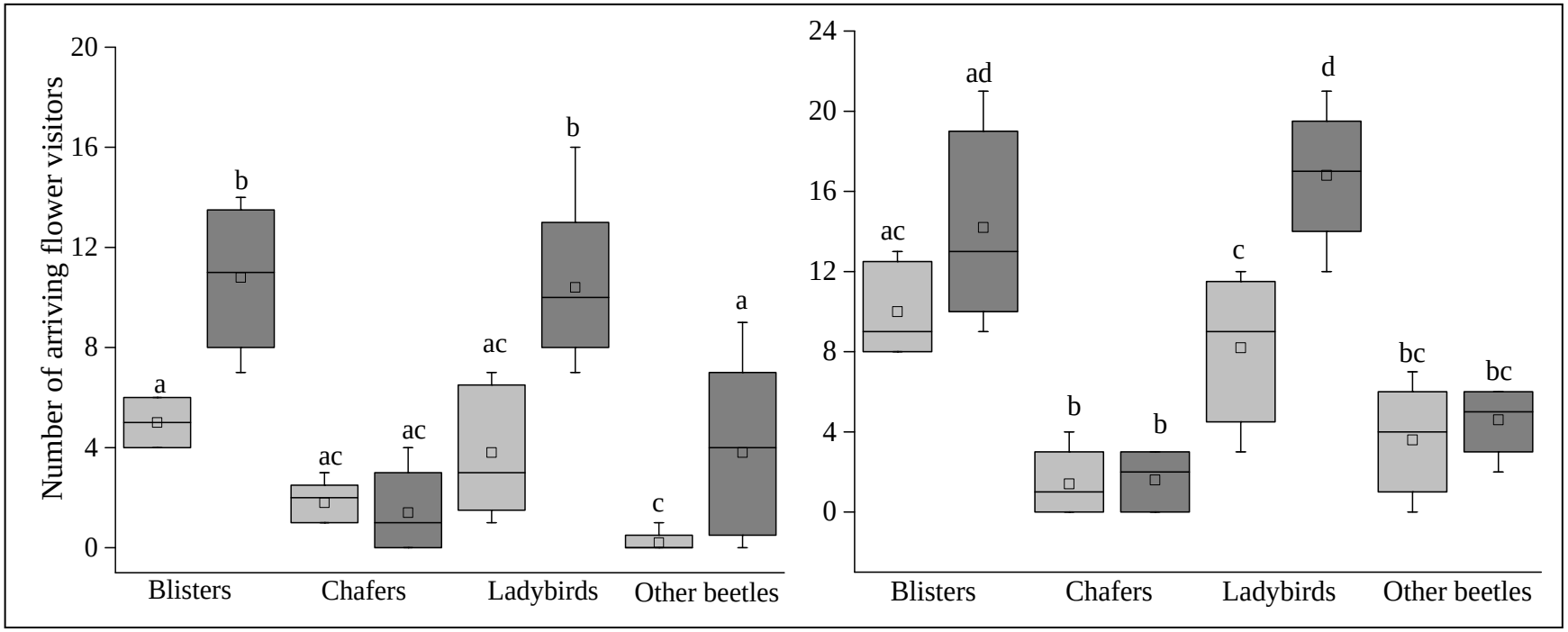


Figure 7: The Number of Arriving Beetles to Flowers of *O. gratissimum* (left) and *A. conyzoides* (right) on the Invaded (light grey) and Uninvaded (grey) Sites per 15-min Observation Period. Different Letters on Bars Indicate Significant Differences by Tukey's HSD Test at $p = 0.05$. Boxplots Show the Mean (Square within Boxes), 25% and 75% Quartile Ranges and Whiskers show the 5^{th} and 95^{th} Percentiles

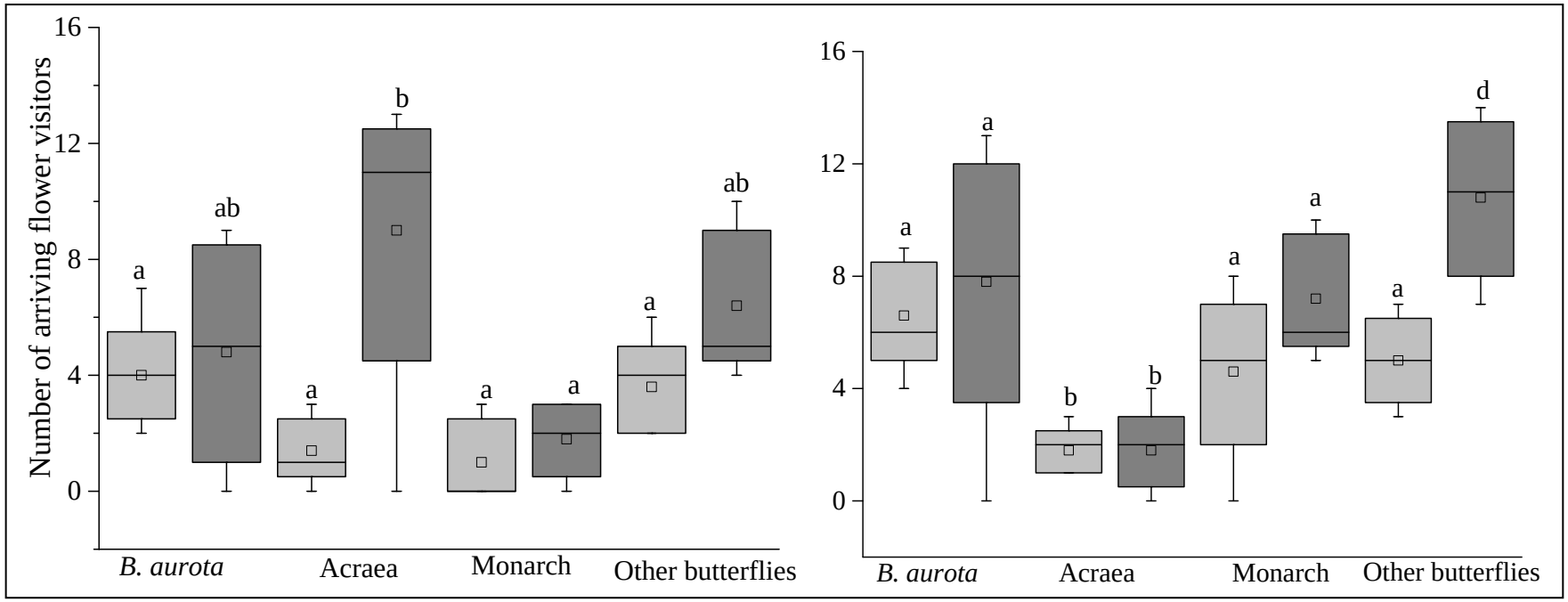


Figure 8: The Number of Arriving Butterflies to Flowers of *O. gratissimum* (left panels) and *A. conyzoides* (right panels) on the Invaded (light grey) and Uninvaded (grey) Sites per 15-min observation period. Different Letters on Bars Indicate Significant Differences by Tukey's HSD Test at $p = 0.05$. Boxplots Show the Mean (square within boxes), 25% and 75% Quartile Ranges and Whiskers Show the 5th and 95th Percentiles

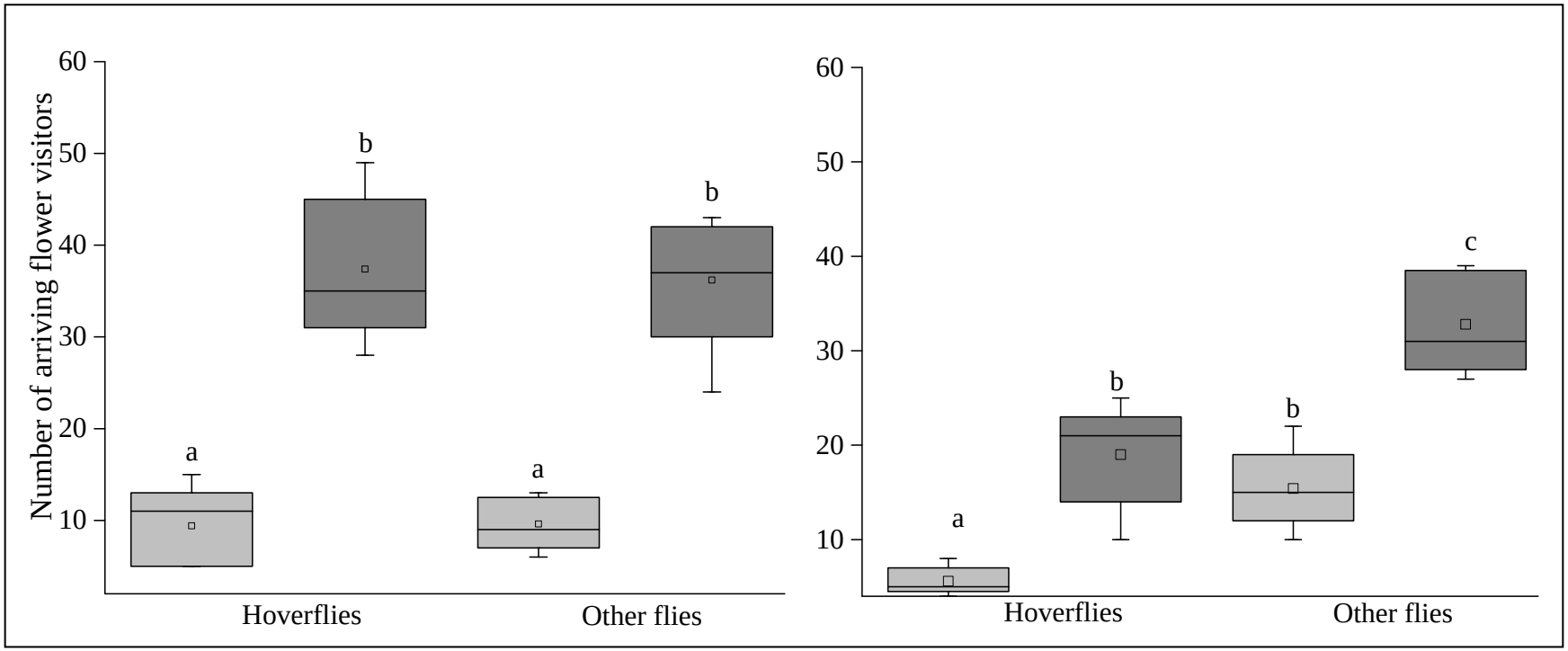


Figure 9: The Number of Arriving Flies to Flowers of *O. gratissimum* (left) and *A. conyzoides* (right) on the Invaded (light grey) and Uninvaded (grey) sites per 15-min Observation Period. Different Letters on bars Indicate Significant Differences by Tukey's HSD Test at $p = 0.05$. Boxplots Show the Mean (square within boxes), 25% and 75% Quartile Ranges and Whiskers Show the 5^{th} and 95^{th} Percentiles

4.1.4 Duration of Visits and Flower Visitation Rate in Invaded and Uninvaded Sites

Duration of visits and visitation rate of insect taxonomic groups, the bees, ants and wasps, beetles, butterflies and flies to flowers of target plants was significant different between the invaded and uninvaded quadrats but not the visitation rate of butterflies and flies to flowers of *A. conyzoides* (Table 6). In general, insects spent longer time interacting with individual flowers of both target species on the uninvaded quadrats, the duration of visits of *A. mellifera* to flowers of *O. gratissimum* ($p = 0.0216$) and *A. conyzoides* ($p = 0.0122$) on the uninvaded quadrats was about two and three times the duration of visits on the invaded quadrats, respectively (Fig. 10). The duration of visits of ladybird beetles ($p = 0.0122$, Fig. 11), other beetles ($p = 0.0119$, Fig. 11), acraea butterflies ($p = 0.0117$, Fig. 12), other butterflies ($p = 0.0022$, Fig. 12) and hoverflies ($p = 0.0122$, Fig. 13) to flowers of *O. gratissimum* on the uninvaded quadrats was about twice the duration of visits on the invaded quadrats.

Also, other flies (*O. gratissimum*: $p = 0.0119$, Fig. 13), ladybird beetles (*A. conyzoides*: $p = 0.0122$, Fig. 11), and other butterflies (*A. conyzoides*: $p = 0.0122$, Fig. 12) had longer duration of visits to flowers of target plants on the uninvaded quadrats, about three times the duration of visits on the invaded quadrats. Moreover, the duration of visits of blister beetles ($p = 0.0122$, Fig. 11) and other flies ($p = 0.0122$, Fig. 13) to flowers of *A. conyzoides* on the uninvaded quadrats was twice the duration of visits on the invaded quadrats, and that of hoverflies ($p = 0.0121$, Fig. 13) on the uninvaded quadrats was four times the duration of visits on the invaded quadrats.

The visitation rate by *A. mellifera* to the flowers of *O. gratissimum* ($p = 0.0012$) and *A. conyzoides* ($p = 0.0001$) on the uninvaded quadrats was about twice the visitation rate of *A. mellifera* on the invaded quadrats (Fig. 14). Also, the visitation rate of blister beetles ($p = 0.0119$), ladybird beetles ($p = 0.0032$) and acraea butterflies ($p = 0.0432$) to flowers of *O. gratissimum,* and lady beetles ($p = 0.0367$) to flowers of *A. conyzoides* on the uninvaded quadrats was about twice as high compared to that on the invaded quadrats (Fig. 15 and Fig. 16). Moreover, the visitation of rate of hoverflies ($p = 0.0178$) to flowers of *O. gratissimum* on the uninvaded quadrats was three times the visitation rate on the invaded quadrats (Fig. 17). In contrast, the visitation rate of flies ($H = 6.91$, $df = 3$, $p > 0.05$) and butterflies ($H = 16.13$, $df = 7$, $p < 0.05$) to flowers of *A. conyzoides* did not differ statistically between the invaded and uninvaded quadrats (Table 6).

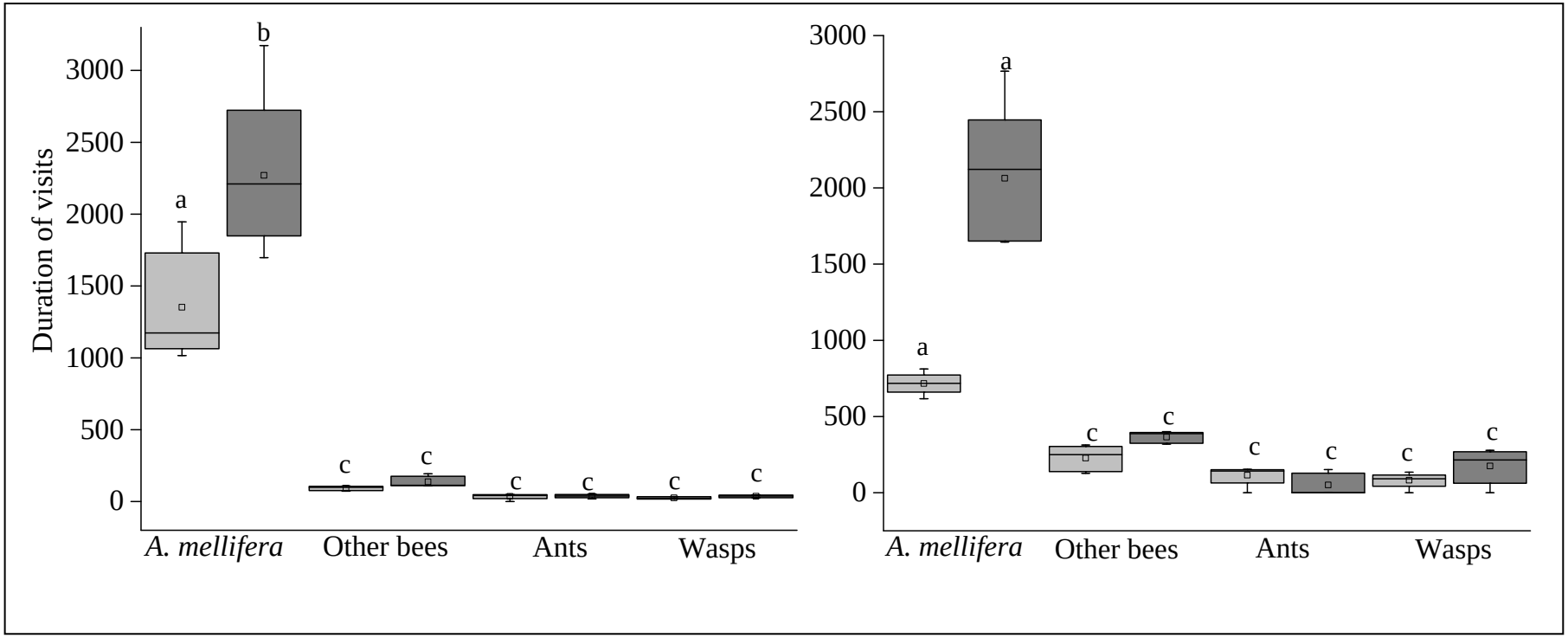


Figure 10: Duration of Visits (in second) of Hymenoptera to Flowers of *O. gratissimum* (left) and *A. conyzoides* (right) on the Invaded (light grey) and Uninvaded (grey) Sites per 15-min observation period. Different letters on bars indicate significant differences by Mann–Whitney pairwise test at p = 0.05. Boxplots show the Median (horizontal line within boxes), 25% and 75% Quartile Ranges and Whiskers Indicate the 5th and 95th Percentiles

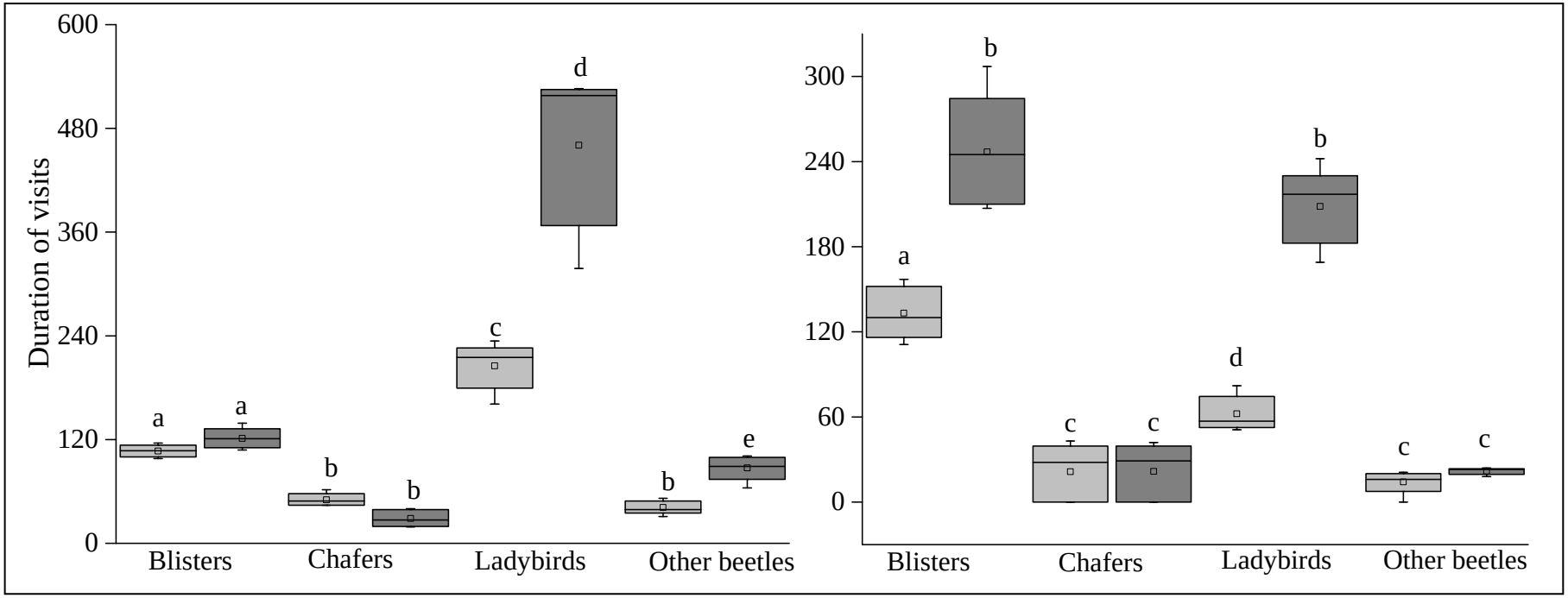


Figure 11: Duration of Visits (in second) of Beetles to Flowers of *O. gratissimum* (left) and *A. conyzoides* (right) on the Invaded (light grey) and Uninvaded (grey) sites per 15-min Observation Period. Different Letters on Bars Indicate Significant Differences by Mann–Whitney Pairwise Test at p = 0.05. Boxplots Show the Median (horizontal line within boxes), 25% and 75% Quartile Ranges and Whiskers Indicate the 5th and 95th Percentiles

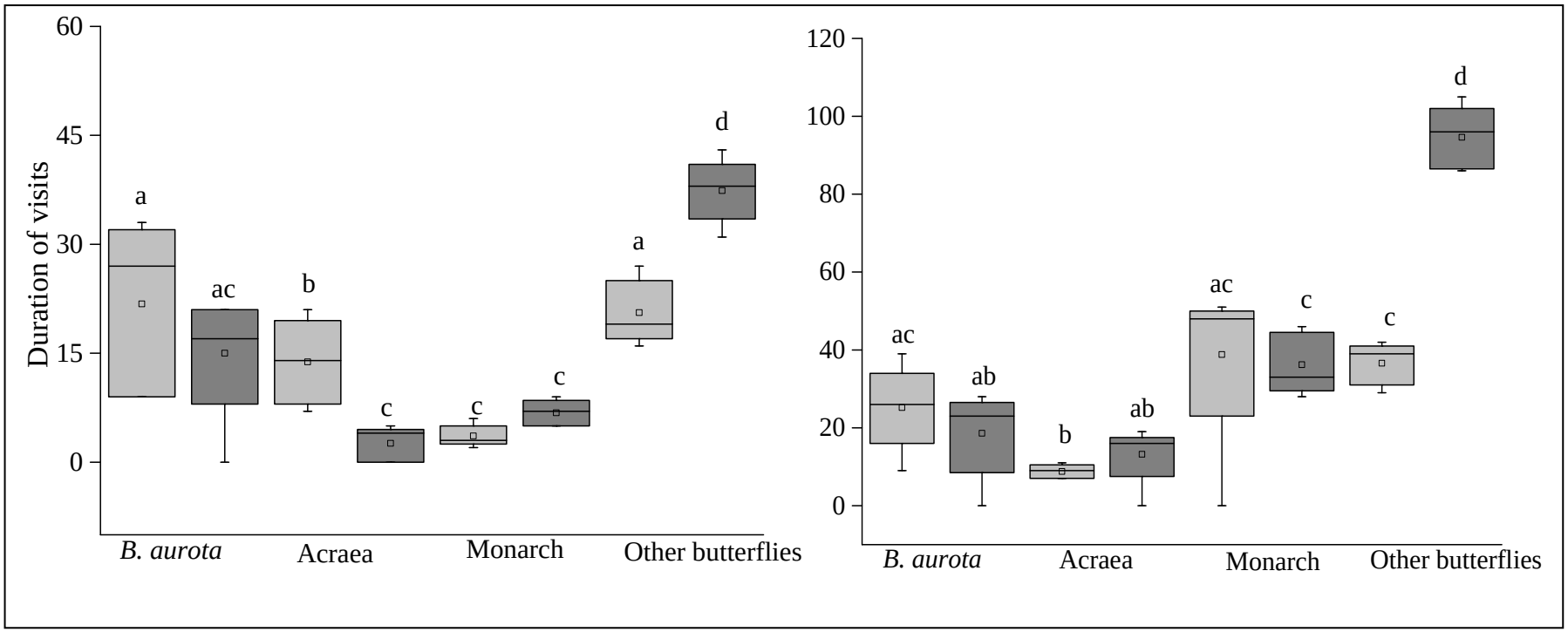


Figure 12: Duration of Visits (in second) of Butterflies to Flowers of *O. gratissimum* (left) and *A. conyzoides* (right) on the Invaded (light grey) and Uninvaded (grey) Sites per 15-min Observation Period. Different Letters on Bars Indicate Significant Differences by Mann–Whitney Pairwise Test at p = 0.05. Boxplots Show the Median (horizontal line within boxes), 25% and 75% Quartile Ranges and Whiskers Indicate the 5th and 95th Percentiles

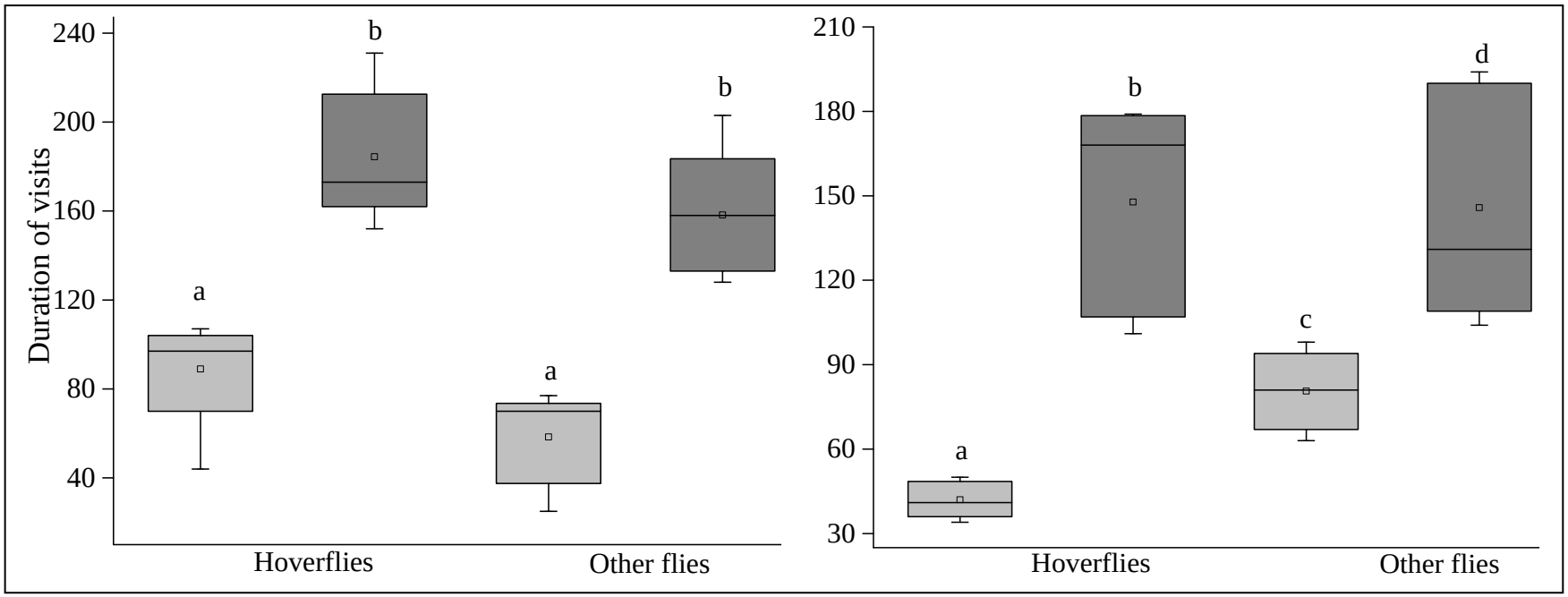


Figure 13: Duration of Visits (in second) of Flies to Flowers of *O. gratissimum* (left) and *A. conyzoides* (right) on the Invaded (light grey) and Uninvaded (grey) sites per 15-min Observation Period. Different Letters on Bars Indicate Significant Differences by Mann–Whitney Pairwise Test at p = 0.05. Boxplots Show the Median (horizontal line within boxes), 25% and 75% Quartile Ranges and Whiskers Indicate the 5th and 95th Percentiles

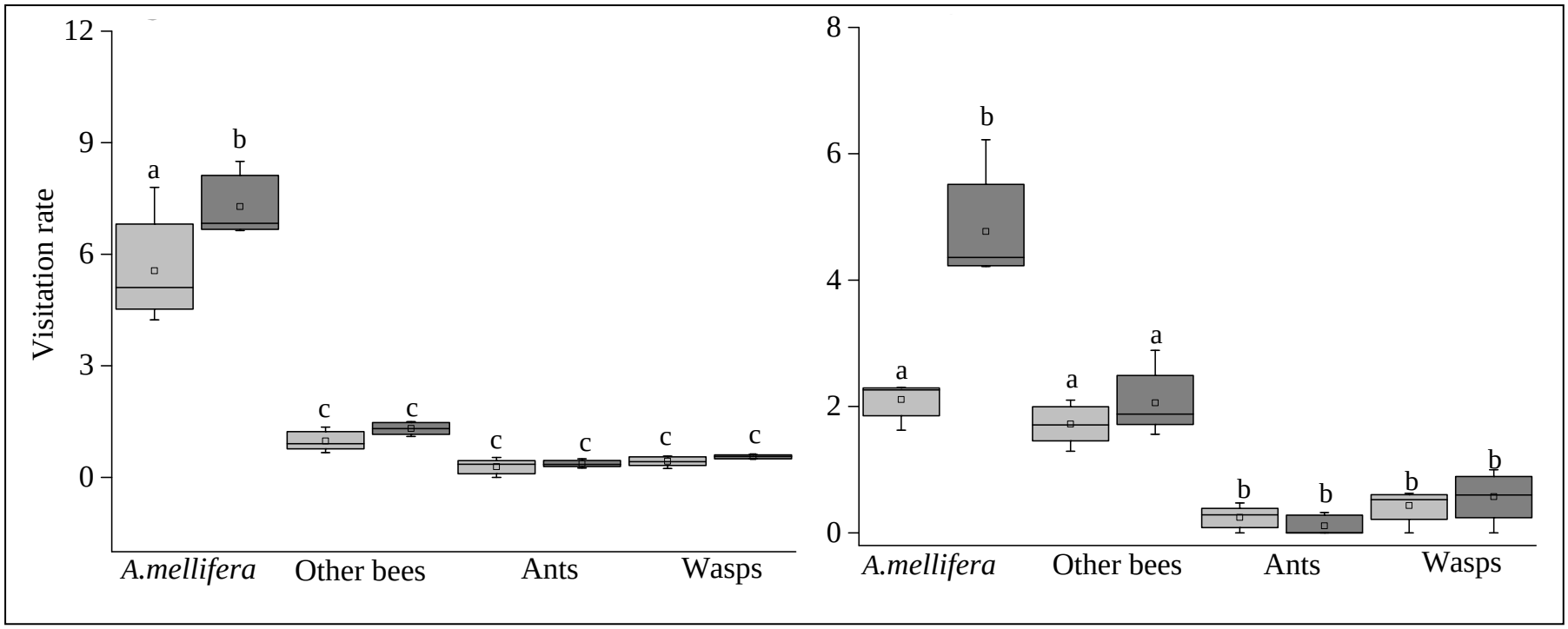


Figure 14: Visitation rate of Hymenoptera to flowers of *O. gratissimum* (left) and *A. conyzoides* (right) on the invaded (light grey) and Uninvaded (grey) Sites per 15-min Observation Period. Different Letters on Bars Indicate Significant Differences by Mann–Whitney Pairwise Test at p = 0.05. Boxplots Show the Median (horizontal line within boxes), 25% and 75% Quartile Ranges and Whiskers Indicate the 5th and 95th Percentiles

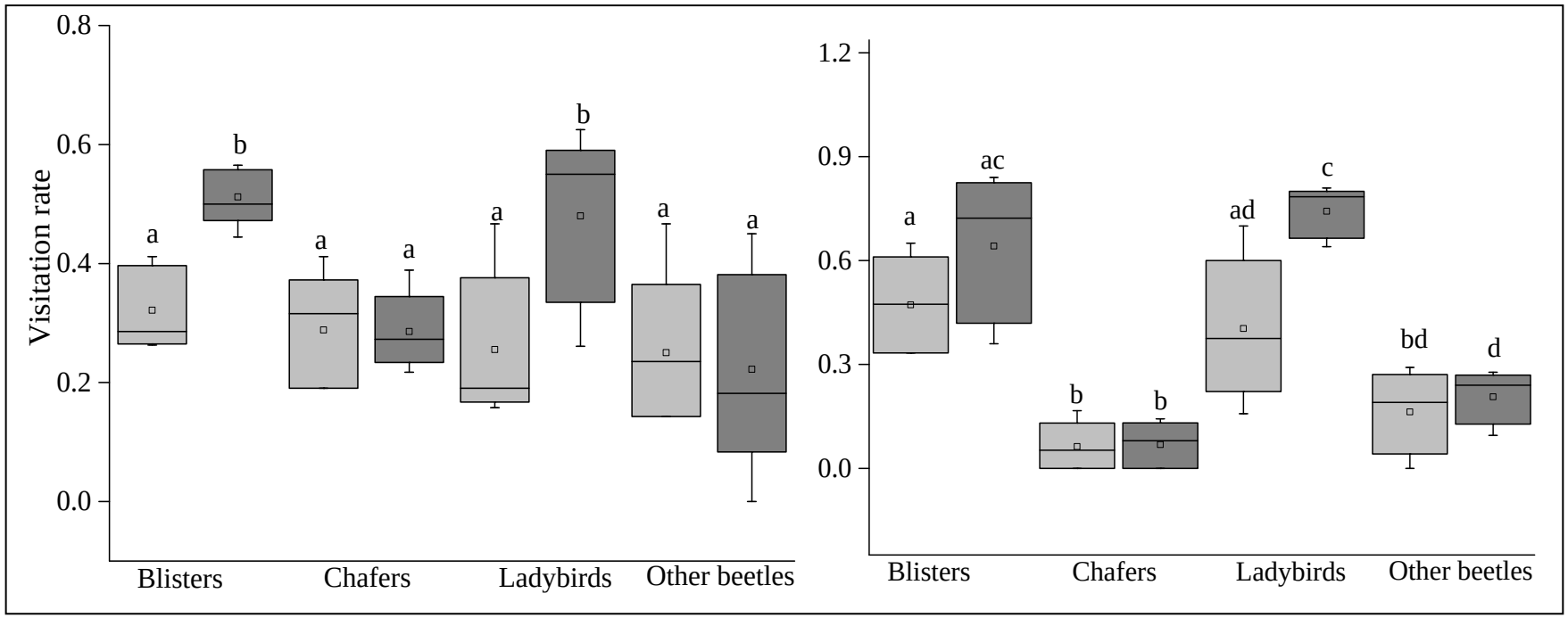


Figure 15: Visitation Rate of Beetles to Flowers of *O. gratissimum* (left) and *A. conyzoides* (right) on the Invaded (light grey) and Uninvaded (grey) Sites per 15-min Observation Period. Different Letters on Bars Indicate Significant Differences by Mann–Whitney Pairwise Test at p = 0.05. Boxplots Show the Median (horizontal line within boxes), 25% and 75% Quartile Ranges and Whiskers Indicate the 5th and 95th Percentiles

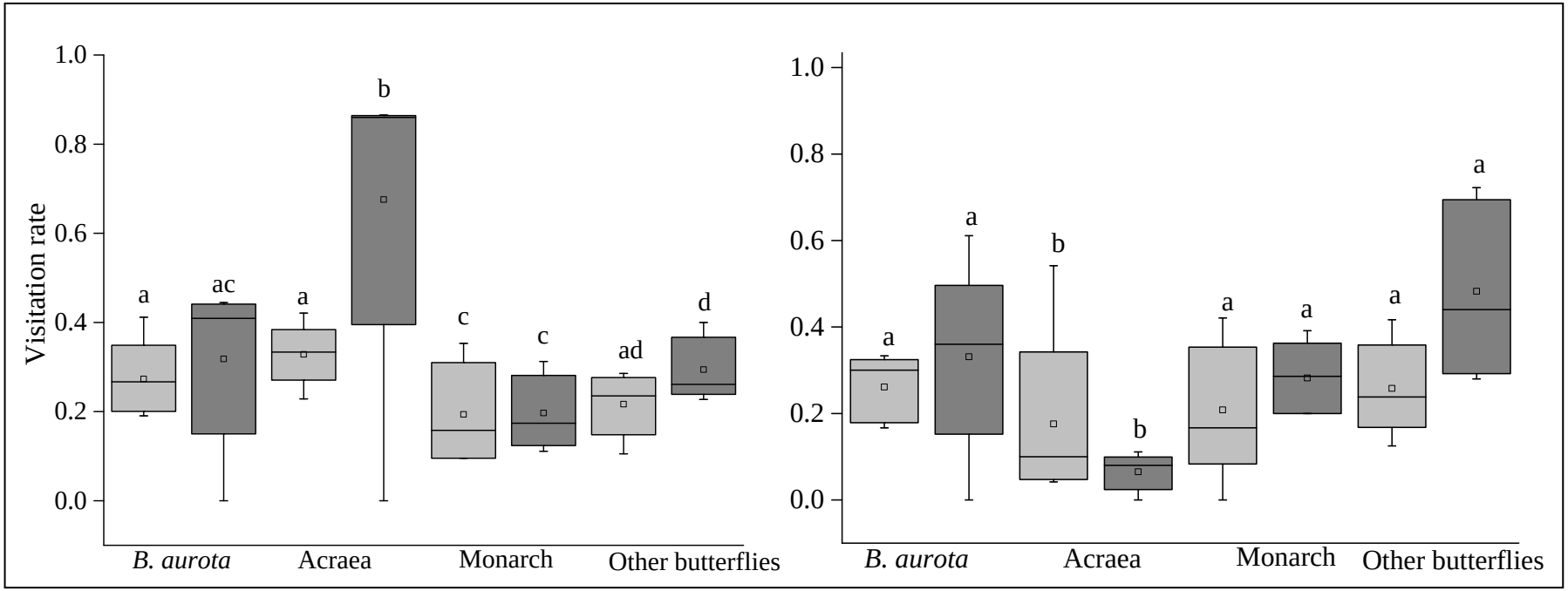


Figure 16: Visitation Rate of Butterflies to Flowers of *O. gratissimum* (left) and *A. conyzoides* (right) on the Invaded (light grey) and Uninvaded (grey) sites per 15-min Observation Period. Different Letters on Bars Indicate Significant Differences by Mann–Whitney Pairwise test at p = 0.05. Boxplots Show the Median (horizontal line within boxes), 25% and 75% Quartile Ranges and Whiskers Indicate the 5th and 95th Percentiles

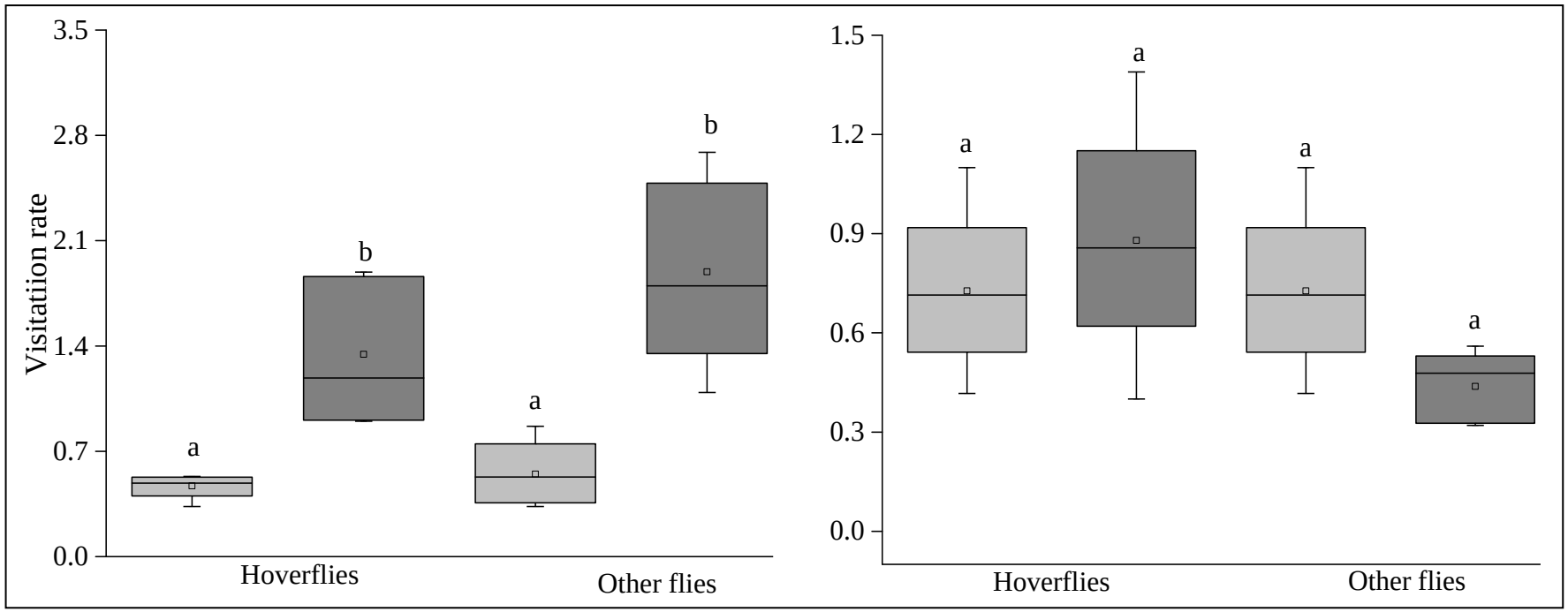
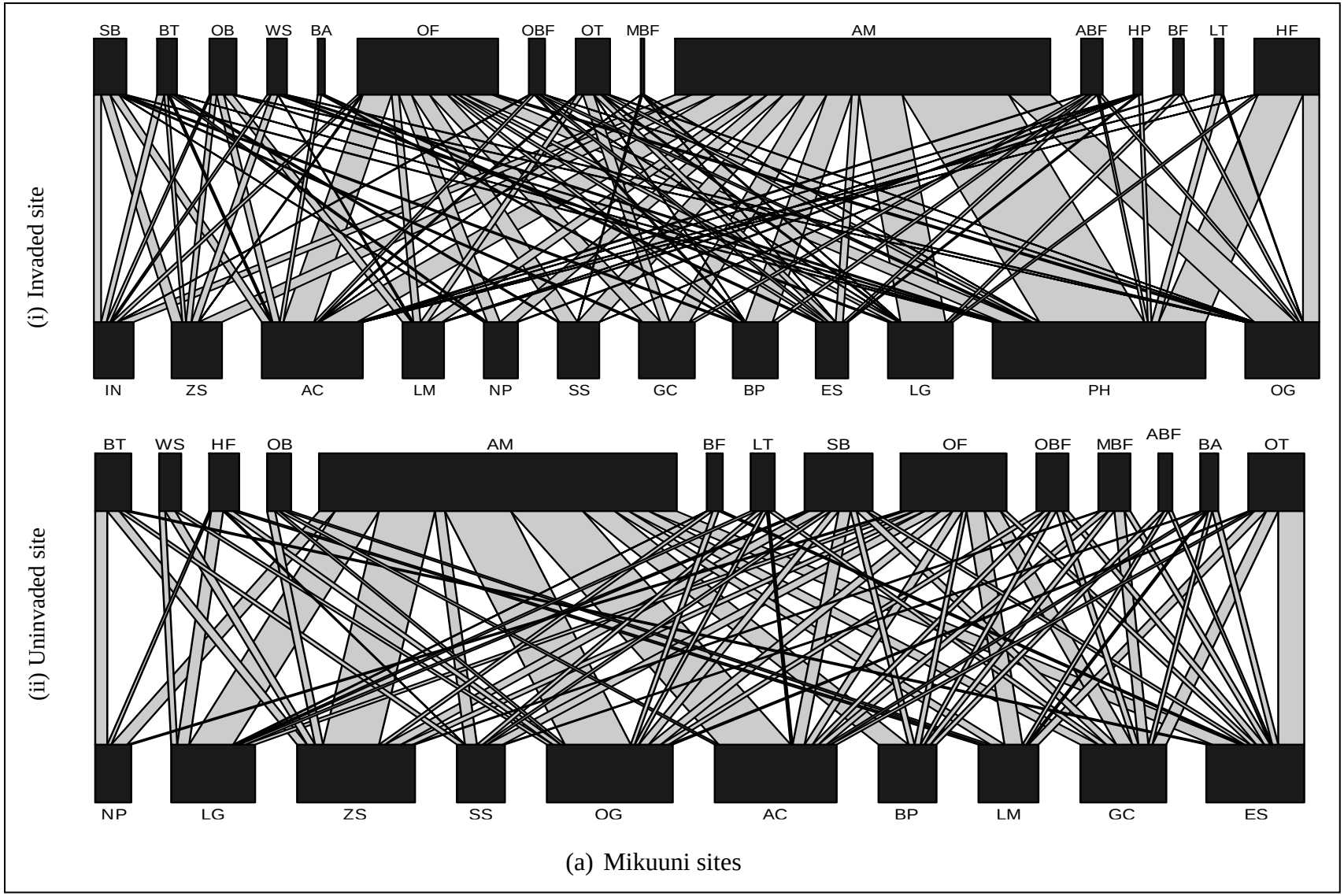


Figure 17: Visitation Rate of Flies to Flowers of *O. gratissimum* (left) and *A. conyzoides* (right) on the Invaded (light grey) and Uninvaded (grey) Sites per 15-min Observation Period. Different Letters on Bars Indicate Significant Differences by Mann–Whitney Pairwise Test at p = 0.05. Boxplots Show the Median (horizontal line within boxes), 25% and 75% Qartile Ranges and Whiskers Indicate the 5th and 95th Percentiles

4.1.5 Visitation Network of Plant–Flower Visitors

A total of 1103 and 987 interactions between co-flowering plant species and flower visiting-insects on the invaded sites and 429 and 555 interactions on the uninvaded sites were recorded at Tengeru and Mikuuni respectively. The mean number of interactions per site was similar between invaded and uninvaded sites (766 and 771 respectively). *P. hysterophorus* interacted with 27% and 63% of co-flowering invasive (i.e. *P. hysterophorus, A. conyzoides*, *Bidens pilosa* and *Nicandra physaloides*) and native plant species respectively via the flower visitors. Plant species shared *A. mellifera* as the main flower visitor, which also was the most frequent visitor to *P. hysterophorus* in both sites with a total of 60% of visits. Flower insect visitors visited more than one plant species, however, no plant species seemed to be pushed out of the network in the invaded sites (Fig. 18a, b).

Insect taxa did not differ significantly between the two sites. Although some taxa appeared to alter their visitation patterns subtly in the presence of *P. hysterophorus* – for instance, hoverflies seem to stop visiting other plants and mostly go to *P. hysterophorus* (Fig. 18a, b). Network metrics were similar, regardless of whether the site was invaded or not (connectance and specialisation (Table 7). However, nestedness was higher at both uninvaded sites compared to their corresponding invaded ones, indicating a higher level of randomness in the interactions on the uninvaded sites (Table 7). Generality and links per species are lower on the uninvaded sites as all insect groups showed more generalised behaviour on the invaded sites (interacting with a larger number of plants) (Table 7).



(a) Mikuuni sites

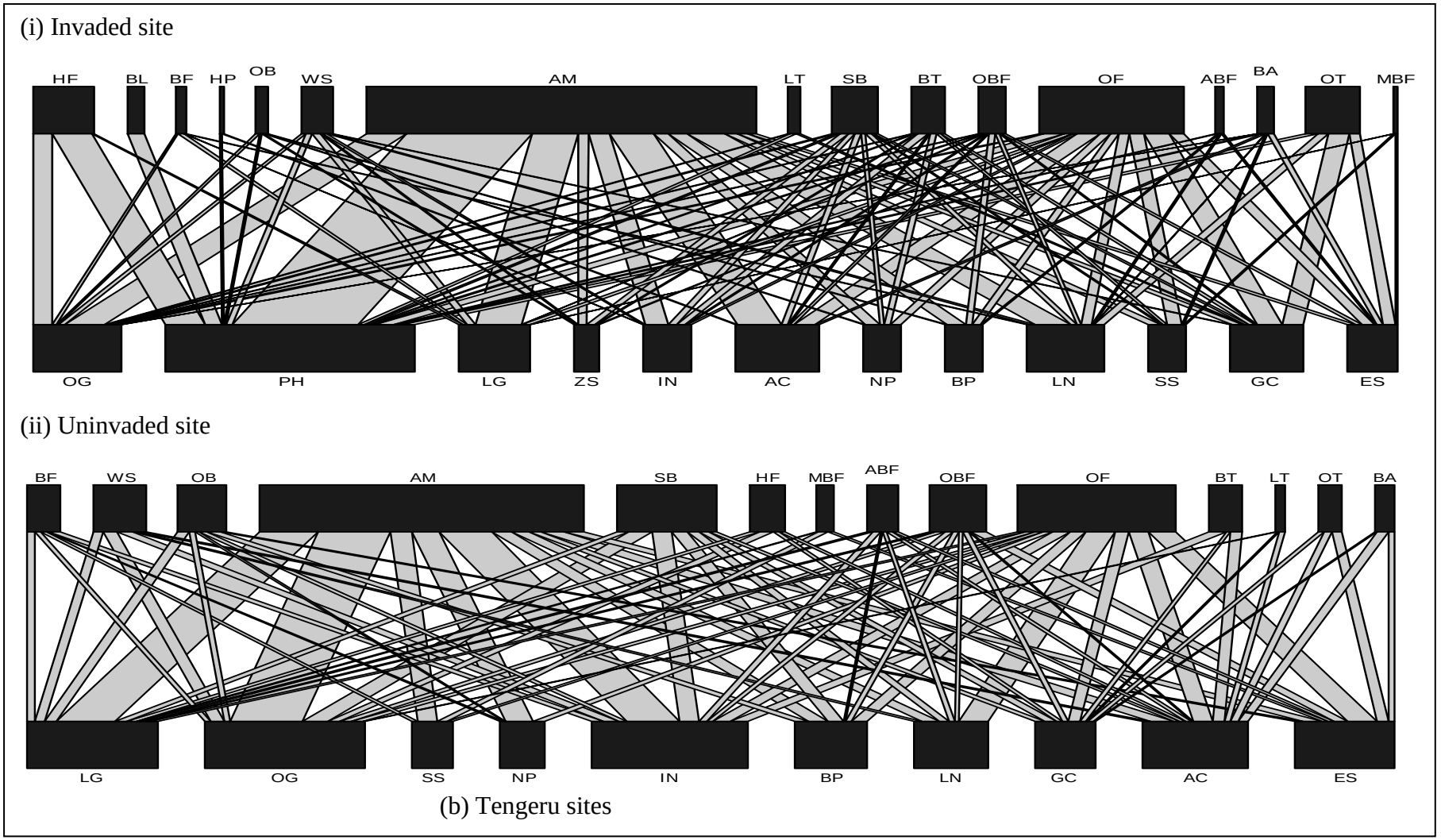


Figure 18: Visitation Web Showing Plant-Flower Visitor Interactions in Invaded and Uninvaded Plots at Mikuuni (a) and Tengeru (b) Study Sites

Black boxes represent flower visiting-insects in the upper level, and plants in the lower level, the width of which indicates the number of visits recorded. Grey links indicate plant-flower visitor interactions, and the visitation frequency is represented by the magnitude of interactions (i.e., breadth of the links). Abbreviations for flower visiting-insects are AM: Apis mellifera, SB: Small bees, OB: Other bees, WS: Wasps, BA: *Balenois aurota*, ABF: Acraea butterflies, MBF: Monarch butterflies, OBF: Other butterflies, HP: Hemiptera, BT, Blister beetles, LT: Ladybird beetles, OT: Other beetles, HF: Hoverflies, BL: Blowflies, BF: Bee flies, OF: Other flies, whereas plants are PH: *Parthenium hysterophorus*, OG: *Ocimum gratissimum*, AC: *Ageratum conyzoides*, GC: *Gutenbergia cordifolia*, IN: *Ipomoea nil*, LG: *Leucas grandis*, LN: *Leonotis nepetifolia*, LM: *Leonotis molis*, NP: *Nicandra physaloides*, SS: *Sphaeranthus suaveolens*, BP: *Bidens pilosa*, ES: Emilia sp, and ZS: *Zehneria scabra*.

Table 7: Network-Level Metrics for the Invaded and Uninvaded Study Sites Based on the Number of Visits by Flower Visitors to each Plant Species

Network-level metrics	Tengeru site		Mikuuni site	
	Invaded	Uninvaded	Invaded	Uninvaded
Connectance	0.5	0.5	0.5	0.5
Nestedness	25.4	31.8	29.7	41.9
Specialization H_2' index	0.2	0.2	0.2	0.2
Generality	7.3	6.8	7.6	6.8
Linkage density	6.1	6.5	6.0	6.1
Links per species	3.3	3.2	3.5	3.2

4.1.6 Effects of Selected Suppressive Plant Species on *P. hysterophorus* Growth Vigour

Overall, *P. hysterophorus* growth vigour was more reduced when its seedlings were grown with *L. purpureus* in all combinations compared to other test plant species. *Parthenium hysterophorus* seedlings had lower stem height, root length, shoot diameter and biomass in mixtures than when grown in monoculture. The stem height (Pot: $F_{(7, 32)} = 9.41$, $p < 0.0001$, Plot: $F_{(7, 32)} = 3.26$, $p = 0.01$, Fig. 19), root length (Pot: $F_{(7, 32)} = 2.78$, $p = 0.0224$, Plot: $F_{(7, 32)} = 11.77$, $p < 0.0001$, Fig. 20) and shoot diameter (Pot: $F_{(7, 32)} = 20.01$, $p < 0.0001$, Plot: $F_{(7, 32)} = 1.67$, $p = 0.0151$, Fig. 21) of *P. hysterophorus* seedlings grown with suppressive plants differed significantly between the number of intercropped suppressive plant species. In the pot experiment, *P. hysterophorus* seedlings stem height was 77% shorter when grown with three suppressive species than when grown with one or two species and in monoculture.

Also, it was > 60% shorter when grown with one (*L. purpureus)* or two (*L. purpureus* and *D. intortum*) suppressive species than when grown alone or with *M. sativa* (Fig. 19). *Parthenium hysterophorus* had > 50% shorter root length when grown with *L. purpureus* and/ or *D. intortum* than when grown in monoculture or with *M. sativa* (Fig. 20). When grown with all three suppressive species *L. purpureus*, *D. intortum* and *M. sativa* together, *P. hysterophorus* root length was 64% shorter than when grown with one or two species, and in monoculture.

Further, when *P. hysterophorus* seedlings were grown with *L. purpureus* in any combination the shoot diameter was reduced by > 62% compared to when grown with other suppressive species and in monoculture (Fig. 21). In the plot experiment, *P. hysterophorus* seedling stem height was > 40% shorter when grown with *L. purpureus* in any combination than when grown with *M. sativa* and/or *D. intortum*, and in monoculture (Fig.19). The root length of *P. hysterophorus* seedlings when grown with two or three suppressive species was > 54% shorter compared to when it was grown with one species or in monoculture (Fig. 20). When *P. hysterophorus* was grown with one suppressive species particularly *L. purpureus*, the root length of seedlings was 45% shorter compared to when grown alone or with either *M. sativa* or *D. intortum*. Moreover, *P. hysterophorus* shoot diameter was > 38% smaller when grown with two or three suppressive plant species than when grown in monoculture or with one suppressive species (Fig. 21).

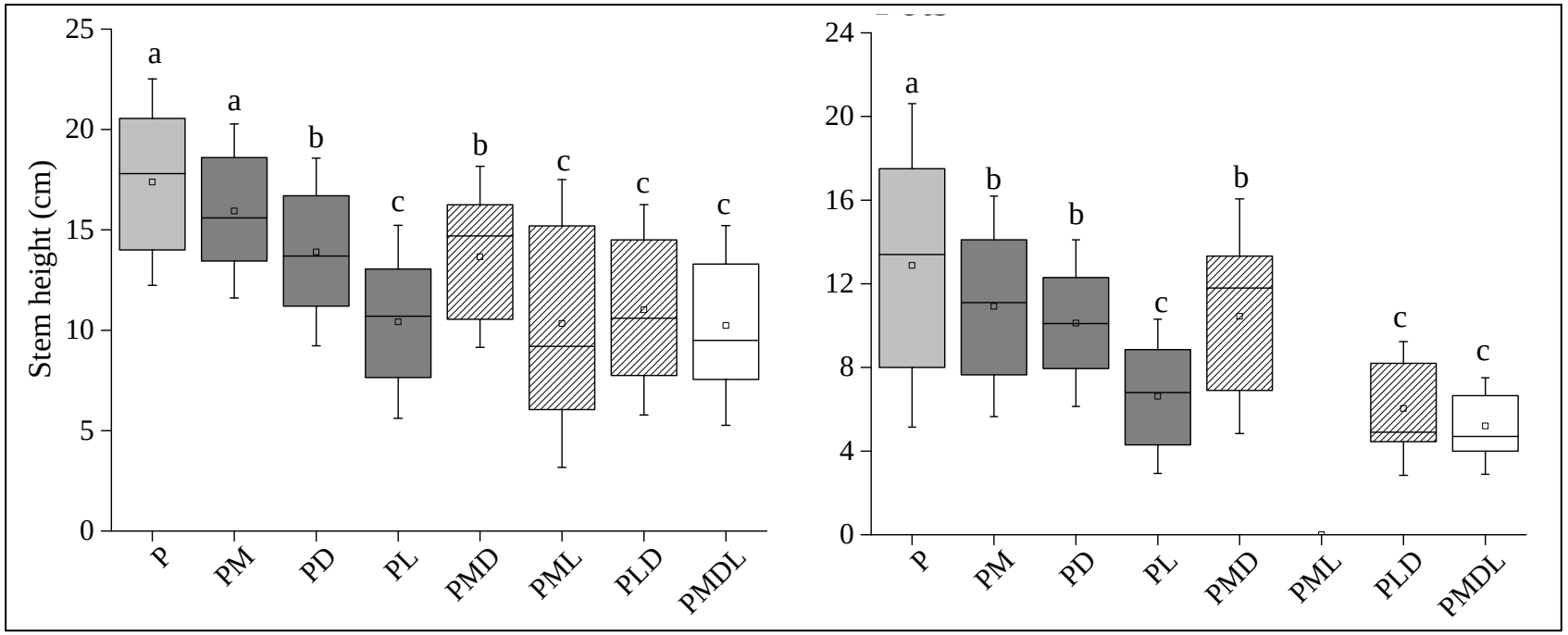


Figure 19: Mean (±SD) Stem Height of *P. hysterophorus* Seedlings when Grown Alone (light grey box), and with One (dark grey boxes), Two (dashed boxes) or Three (white box) Suppressive Plant Species in Plot (left panels) and Pot (right panels) Experiments. Boxes with Dissimilar Letters are Significantly Different by Tukey's HSD Test at $p \leq 0.05$. Boxplots Show the Mean (square within boxes), 25% and 75% Quartile Ranges and whiskers Show Standard Deviations. *Parthenium hysterophorus* Seedlings in Planting Mixture PML in Pot Experiment Died. P = *P. hysterophorus*, M = *M. sativa*, D = *D. intortum* and L = *L. purpureus*

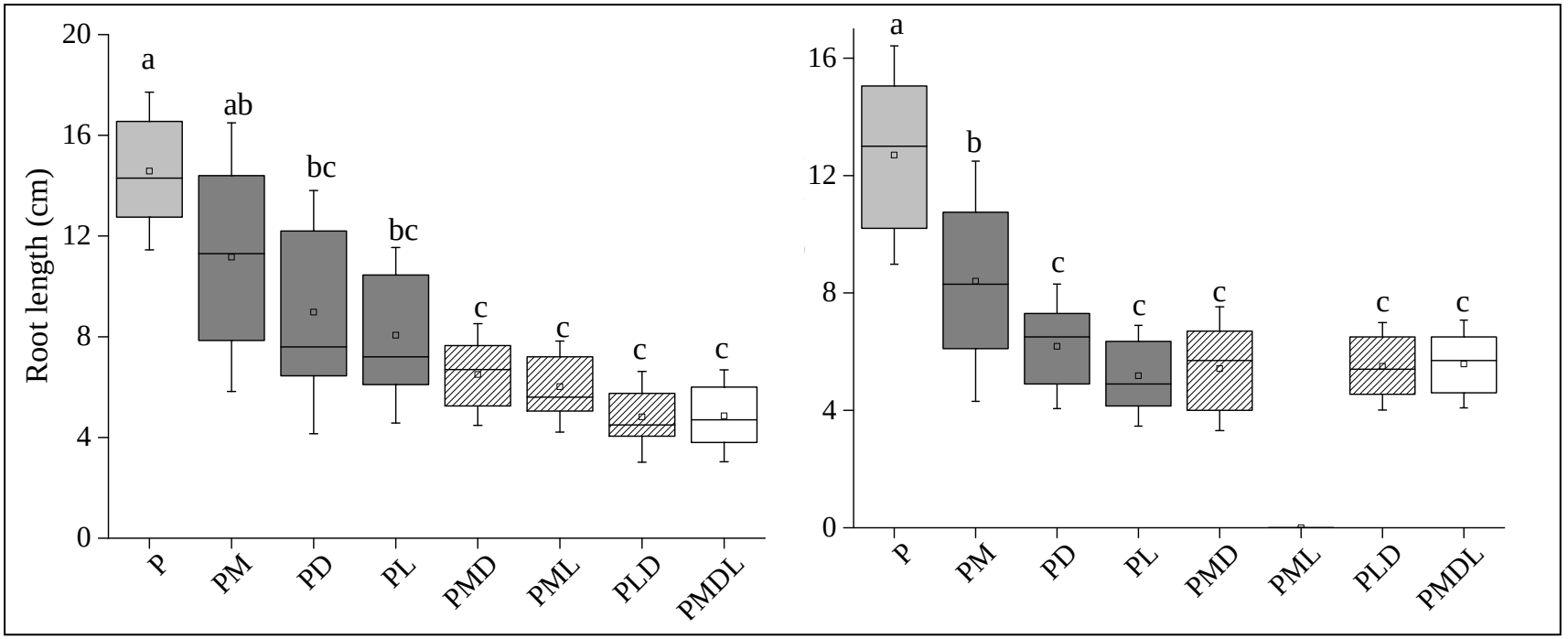


Figure 20: Mean (±SD) Root Length of *P. hysterophorus* Seedlings when Grown Alone (light grey box), and with One (dark grey boxes), two (dashed boxes) or Three (white box) Suppressive Plant Species in Plot (left panels) and Pot (right panels) Experiments. Boxes with Dissimilar Letters are Significantly Different by Tukey's HSD Test at $p \leq 0.05$. Boxplots Show the Mean (square within boxes), 25% and 75% Quartile Ranges and Whiskers Show Standard Deviations. *Parthenium hysterophorus* Seedlings in Planting Mixture PML in pot Experiment Died. P = *P. hysterophorus*, M = *M. sativa*, D = *D. intortum* and L = *L. purpureus*

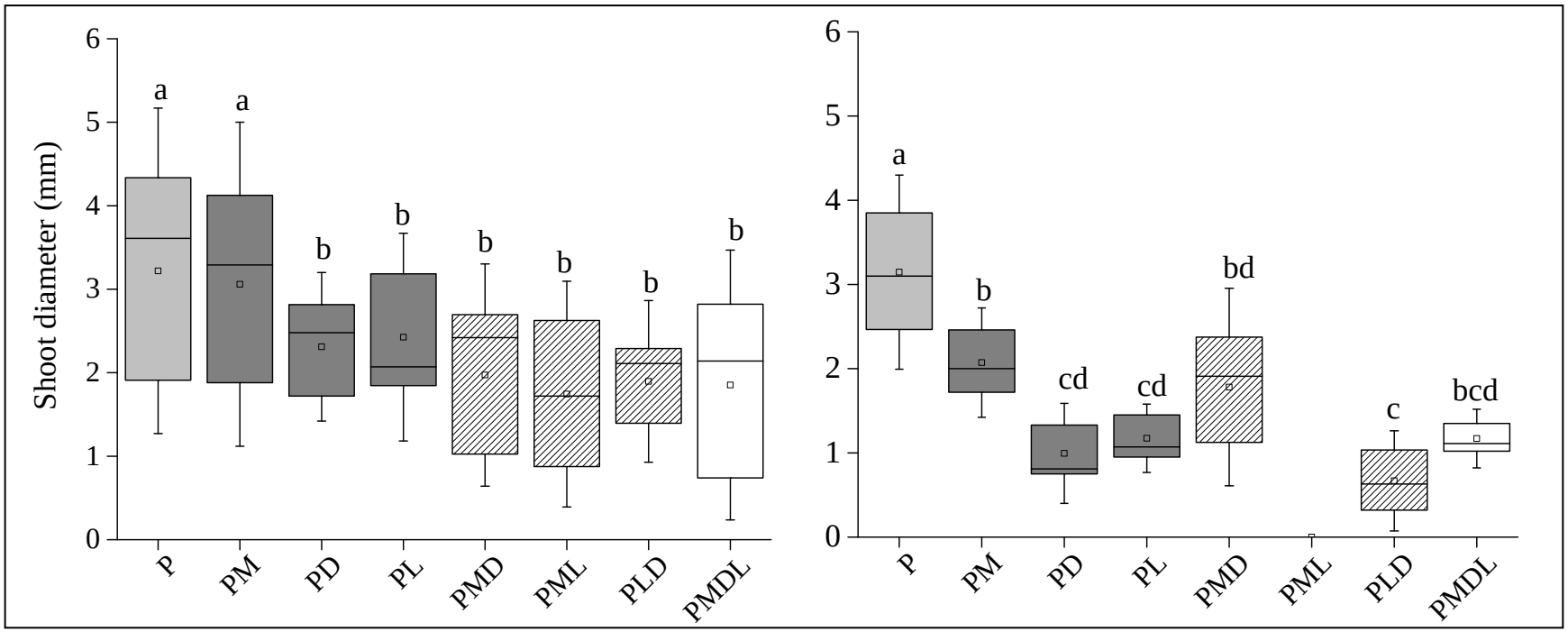


Figure 21: Mean (±SD) Shoot Diameter of *P. hysterophorus* Seedlings when Grown Alone (light grey box), and with One (dark grey boxes), Two (dashed boxes) or Three (white box) Suppressive Plant Species in Plot (left panels) and Pot (right panels) Experiments. Boxes with Dissimilar Letters are Significantly Different by Tukey's HSD Test at $p \leq 0.05$. Boxplots show the Mean (square within boxes), 25% and 75% Quartile Ranges and whiskers Show Standard Deviations. *Parthenium hysterophorus* Seedlings in Planting Mixture PML in Pot Experiment Died. P = *P. hysterophorus*, M = *M. sativa*, D = *D. intortum* and L = *L. purpureus*

Mean aboveground fresh biomass (AFB) (Pot: $F_{(7, 32)} = 13.99$, $p < 0.0001$, Plot: $F_{(7, 32)} = 11.68$, $p < 0.0001$, Fig. 22) and aboveground dry biomass (ADB) (Pot: $F_{(7, 32)} = 10.33$, $p < 0.0001$, Plot: $F_{(7, 32)} = 9.42$, $p < 0.0001$, Fig. 23) of *P. hysterophorus* seedlings differed significantly between planting diversity. Also, mean belowground fresh biomass (BFB) (Pot: $F_{(7, 32)} = 22.78$, $p < 0.0001$, Plot: $F_{(7, 32)} = 3.23$, $p = 0.0105$, Fig. 24) and belowground dry biomass (BDB) (Pot: $F_{(7, 32)} = 15.94$, $p < 0.0001$, Plot: $F_{(7, 32)} = 3.85$, $p = 0.0038$, Fig. 25) differed significantly between different planting diversity. In pots, *P. hysterophorus* AFB and ADB were > 53% lower when grown with suppressive species in either planting combination than when it was grown alone or with *M. sativa* only (Fig. 22 and 23). *Parthenium hysterophorus* BFB was > 55% lower when grown with suppressive species in any planting combination except when grown with *M. sativa* alone or in monoculture (Fig. 24). Moreover, BDB was > 55% lower when *P. hysterophorus* was grown in mixture of *L. purpureus* than when it was grown in monoculture or with other species alone (Fig. 25).

In the plot experiment, *P. hysterophorus* AFB and ADB were > 66% lower when grown with two (*L. purpureus* and *D. intortum*) or three suppressive species than when grown in monoculture, with one species except *L. purpureus* (Fig. 22 and 23). *Parthenium hysterophorus* BFB was > 55% lower when grown with two (*L. purpureus* and *D. intortum*) or three suppressive species than when grown either in monoculture (Fig. 24). Also, when *P. hysterophorus* was grown with two or three suppressive species, its BDB was > 50% lower than when grown either alone, with *M. sativa* or *D. intortum* (Fig. 25, Table 2). In general, stem height, root length, and shoot diameter of *P. hysterophorus* seedlings were more reduced when grown with *L. purpureus* than other plants species (Table 3).

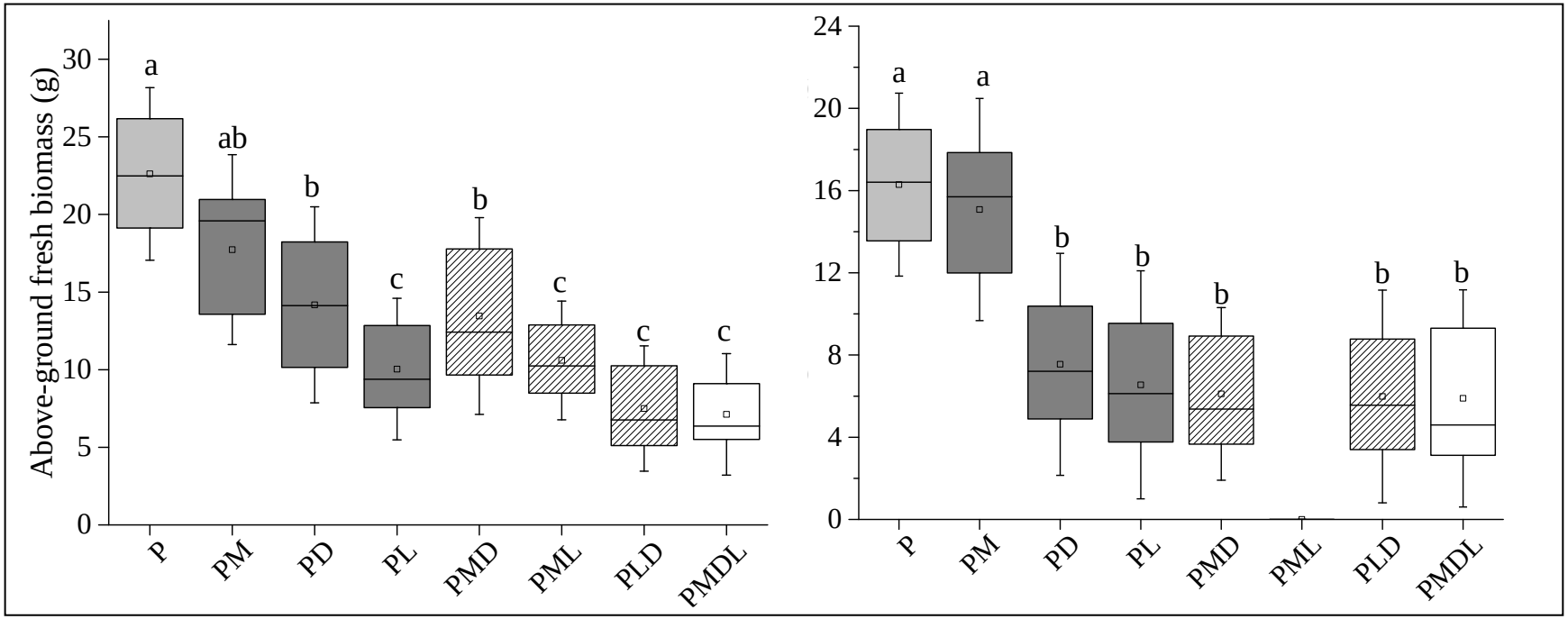


Figure 22: Mean (±SD) Above-Ground Fresh of *P. hysterophorus* Seedlings when Grown Alone (light grey box), and with One (grey boxes), Two (dashed boxes) or Three (white box) Suppressive Plant Species in Plot (left) and Pot (right) Experiments. Boxes with Dissimilar Letters are Significantly Different by Tukey's HSD Test at $p \leq 0.05$. Boxplots show the Mean (square within boxes), 25% and 75% Quartile Ranges and Whiskers Show Standard Deviations. *Parthenium hysterophorus* Seedlings in Planting Mixture PML in Pot Experiment Died. P = *P. hysterophorus*, M = *M. sativa*, D = *D. intortum* and L = *L. purpureus*

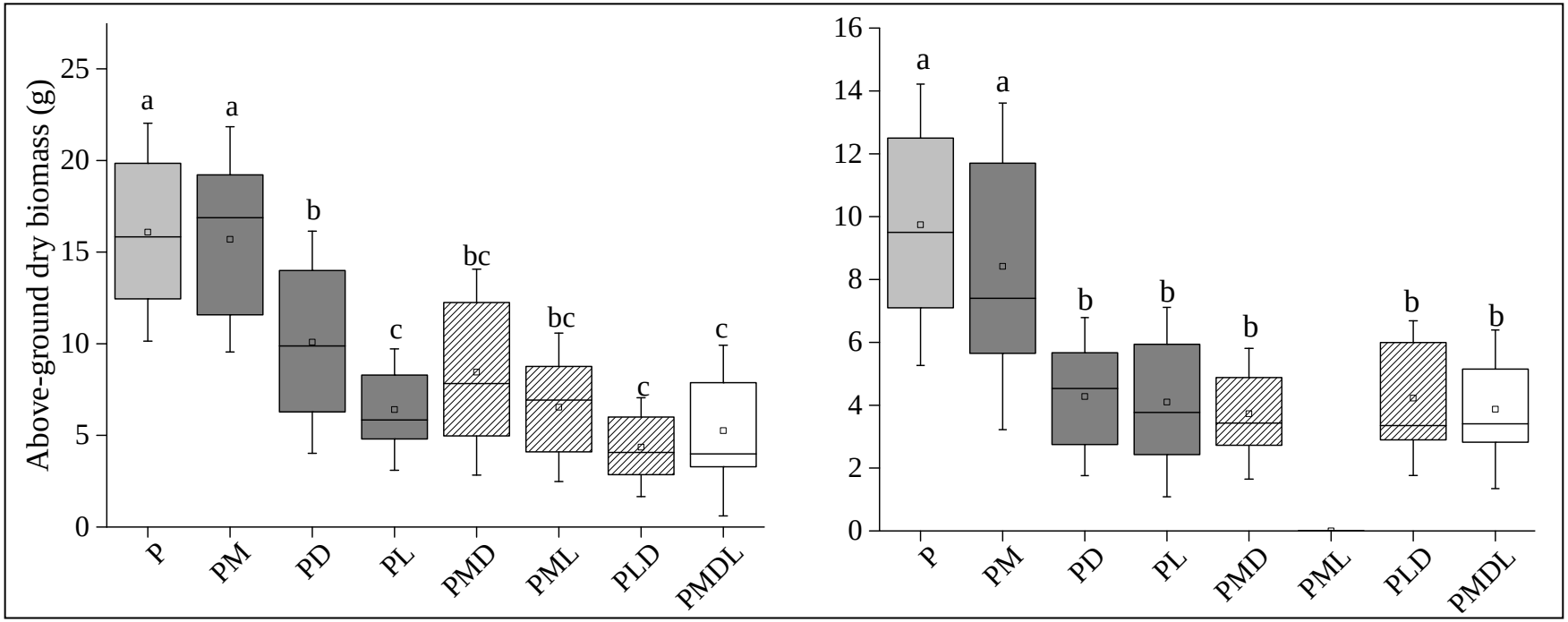


Figure 23: Mean (±SD) Above–Ground Dry Biomass of *P. hysterophorus* Seedlings when Grown Alone (light grey box), and with One (grey boxes), Two (dashed boxes) or Three (white box) Suppressive Plant Species in Plot (left panels) and Pot (right panels) Experiments. Boxes with Dissimilar Letters are Significantly Different by Tukey's HSD test at $p \leq 0.05$. Boxplots show the Mean (square within boxes), 25% and 75% Quartile Ranges and Whiskers Show Standard Deviations. *Parthenium hysterophorus* Seedlings in Planting Mixture PML in Pot Experiment Died. P = *P. hysterophorus*, M = *M. sativa*, D = *D. intortum* and L = *L. purpureus*

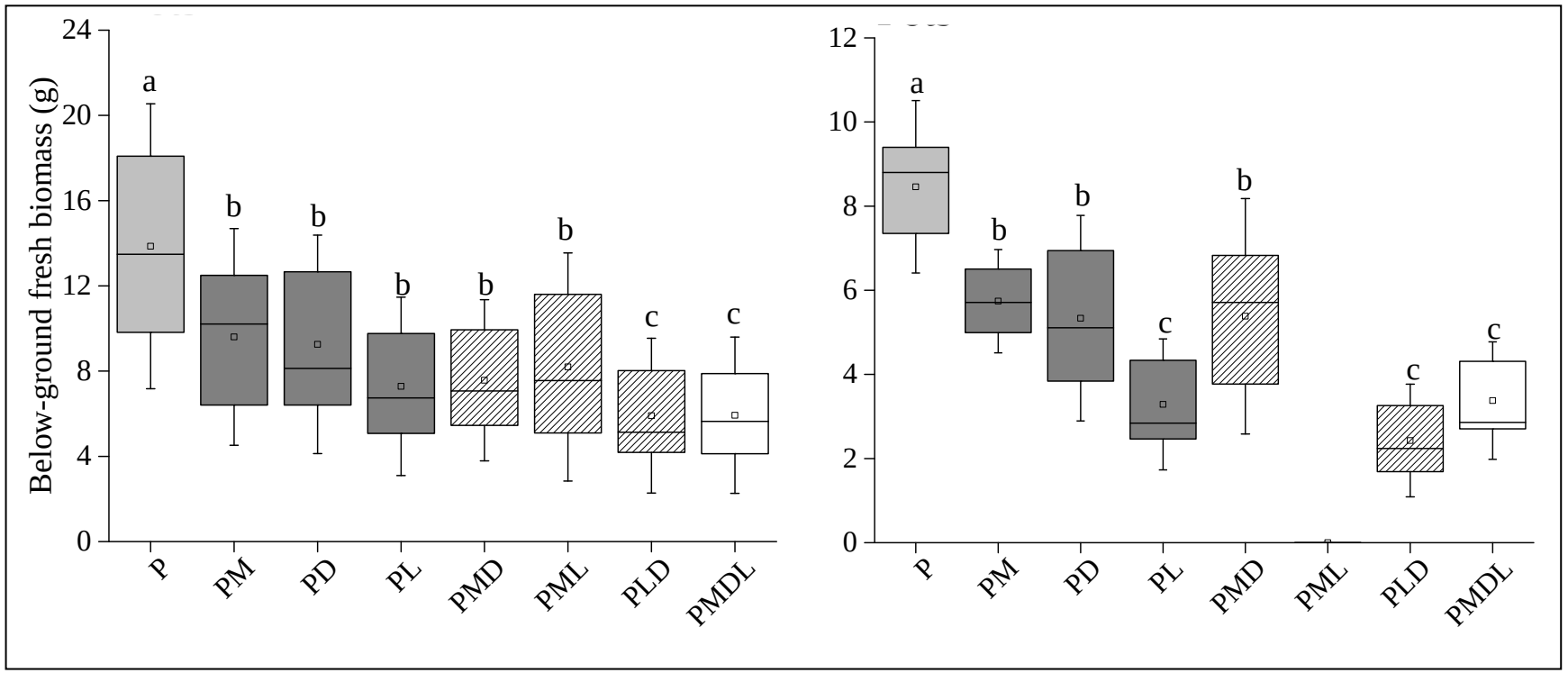


Figure 24: Mean (±SD) Below–Ground Fresh of *P. hysterophorus* Seedlings When Grown Alone (light grey box), and with One (grey boxes), Two (dashed boxes) or Three (white box) Suppressive Plant Species in Plot (left) and Pot (right) Experiments. Boxes with Dissimilar Letters are Significantly Different by Tukey's HSD Test at $p \leq 0.05$. Boxplots show the Mean (square within boxes), 25% and 75% quartile ranges and Whiskers Show Standard Deviations. *Parthenium hysterophorus* Seedlings in Planting Mixture PML in Pot Experiment Died. P = *P. hysterophorus*, M = *M. sativa*, D = *D. intortum* and L = *L. purpureus*

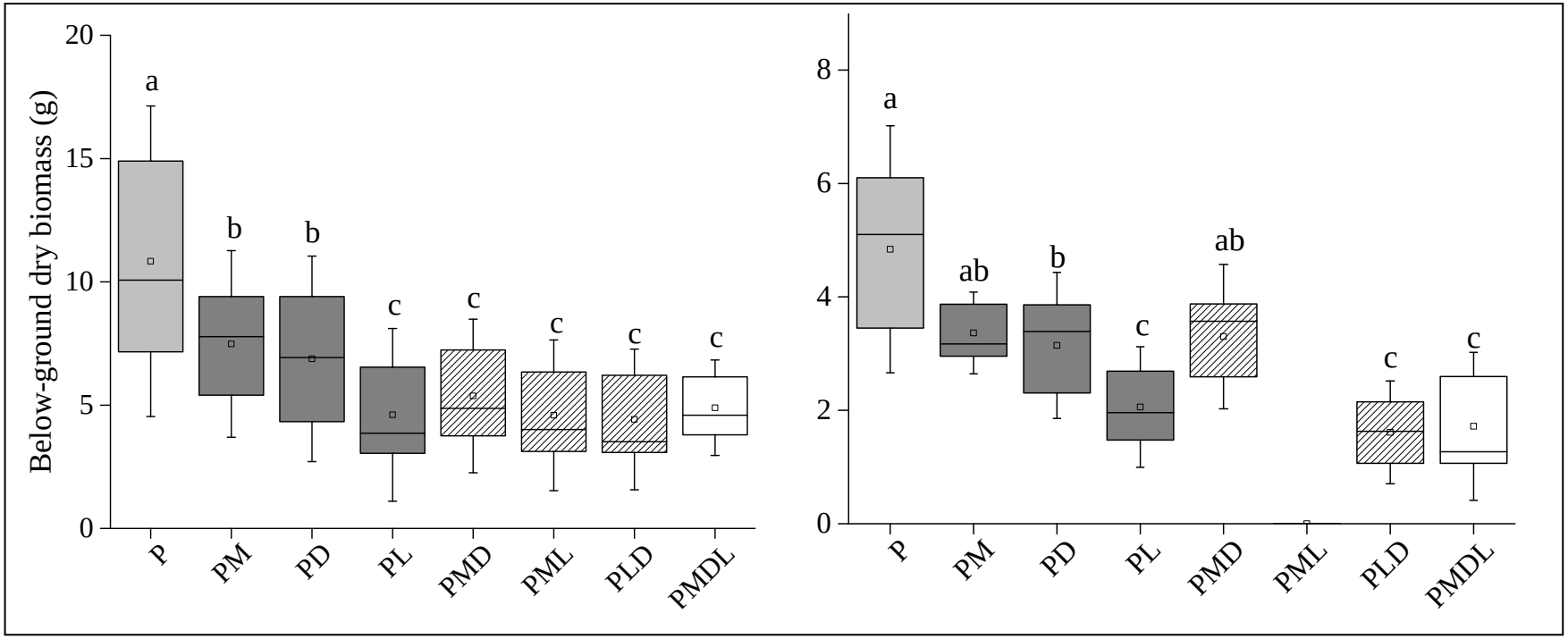


Figure 25: Mean (±SD) Below–Ground Dry Biomass of *P. hysterophorus* Seedlings when Grown Alone (light grey box), and with One (grey boxes), Two (dashed boxes) or Three (white box) Suppressive Plant Species in Plot (left) and Pot (right) Experiments. Boxes with Dissimilar Letters are Significantly Different by Tukey's HSD Test at $p \leq 0.05$. Boxplots show the Mean (square within boxes), 25% and 75% Quartile Ranges and Whiskers Show Standard Deviations. *Parthenium hysterophorus* Seedlings in Planting Mixture PML in Pot Experiment Died. P = *P. hysterophorus*, M = *M. sativa*, D = *D. intortum* and L = *L. purpureus*

In addition, the competition intensity of *P. hysterophorus* in both pot and plot experiments declined (greater and lower values of RCI and RII respectively, refer *eqn 2* and *3*) with increasing suppressive species numbers in planting combination (Table 8). Total fresh biomass (AFB + BFB) of *P. hysterophorus* seedlings was significantly different between suppressive species planting combination in both pot and plot experiments (Pot: $F_{(7, 32)}$ = 23.00, $p < 0.0001$; and Plot: $F_{(7, 32)}$ = 12.38, $p < 0.0001$, Fig. 26). *Parthenium hysterophorus* total fresh biomass was > 53% lower when grown with two or three suppressive species than when it was grown alone or with one species except *L. purpureus* in pots. In plots, total fresh biomass was reduced by 64%, 63% and 53% when grown with three, two (only *L. purpureus* and *D. intortum*) and one (only *L. purpureus*) suppressive species compared to when it was grown in monoculture and in other mixtures (Fig. 26). In each planting combination with *L. purpureus*, suppressive effects on *P. hysterophorus* seedlings biomass was higher compared to when *L. purpureus* was absent (Fig. 27).

Table 8: Relative Competition Intensity (RCI) and Relative Interaction Intensity (RII) for *P. hysterophorus* Seedlings According to Suppressive Plant Diversity in Pot and Plot Experiments

Planting combinations	Pot experiment		Plot experiment	
	RCI	RII	RCI	RII
PM	0.159	-0.086	0.250	-0.143
PD	0.479	-0.315	0.357	-0.218
PL	0.603	-0.431	0.525	-0.356
PMD	0.536	-0.366	0.424	-0.269
PML	-	-	0.485	-0.320
PLD	0.660	-0.493	0.632	-0.462
PMDL	0.626	-0.455	0.642	-0.473

P. hysterophorus (P) was grown in mixture with *M. sativa* (M), *D. intortum* (D) and *L. purpureus* (L).

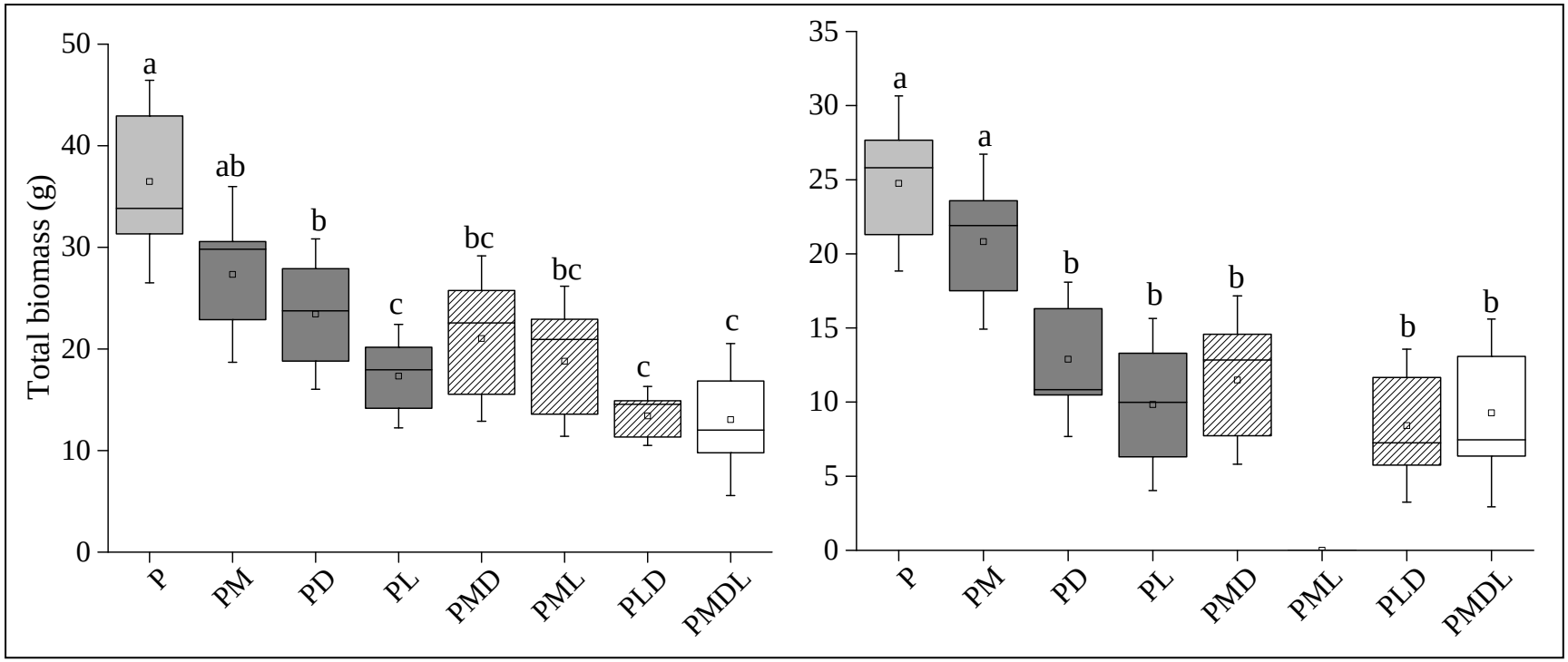


Figure 26: Mean (±SD) Total Fresh Biomass of *P. hysterophorus* Seedlings when Grown Alone (light grey box), and with One (grey boxes), Two (dashed boxes) or Three (white box) Suppressive Plant Species in Plot (left) and Pot (right) Experiments. Boxes with Dissimilar Letters are Significantly Different by Tukey's HSD Test at $p \leq 0.05$. Boxplots show the Mean (square within boxes), 25% and 75% Quartile Ranges and Whiskers Show Standard Deviations. *Parthenium hysterophorus* Seedlings in Planting Mixture PML in Pot Experiment Died. P = *P. hysterophorus*, M = *M. sativa*, D = *D. intortum* and L = *L. purpureus*

4.1.7 Effects of Suppressive Plants on *P. hysterophorus* Leaf Chlorophyll Content

Total leaf chlorophyll content (Chl) of *P. hysterophorus* differed significantly between suppressive plant species diversity in both pot and plot experiments (Pot: $F_{(4, 20)} = 26.87$, $p < 0.0001$ and Plot: $F_{(4, 20)} = 48.36$, $p < 0.0001$, Fig. 27). Suppressive plants negatively affected *P. hysterophorus* Chl when grown with one (except *M. sativa*), two or three suppressive species. In pot experiment, *P. hysterophorus* Chl was reduced by > 75% when grown with three suppressive species compared to when it was grown in other planting combinations or monoculture. However, *P. hysterophorus* Chl was 84% lower when grown with *L. purpureus* alone than when it was grown with *M. sativa, D. intortum* or in monoculture (Fig. 27). Further, in plot experiment, *P. hysterophorus* Chl was reduced by 69% when grown with three suppressive plant species than when it was grown in monoculture or in other planting combinations (Fig. 27).

Furthermore, when *P. hysterophorus* was grown with two or one suppressive species (*D. intortum* or *L. purpureus*), its total Chl was reduced by > 40% than when it was grown alone or with *M. sativa*. In addition, with respect to planting species diversity, *P. hysterophorus* total fresh biomass and leaf Chl were positively correlated both in pot (F = 25.76, r = 0.9151, n = 6, $p = 0.0039$) and plot (F = 51.38, r = 0.9463, n = 6, $p = 0.0004$) experiments (Fig. 28). However, Chl content and biomass decreased with increasing suppressive plant species *M. sativa, D. intortum* and *L. purpureus* diversity in pot and plot (Fig. 28).

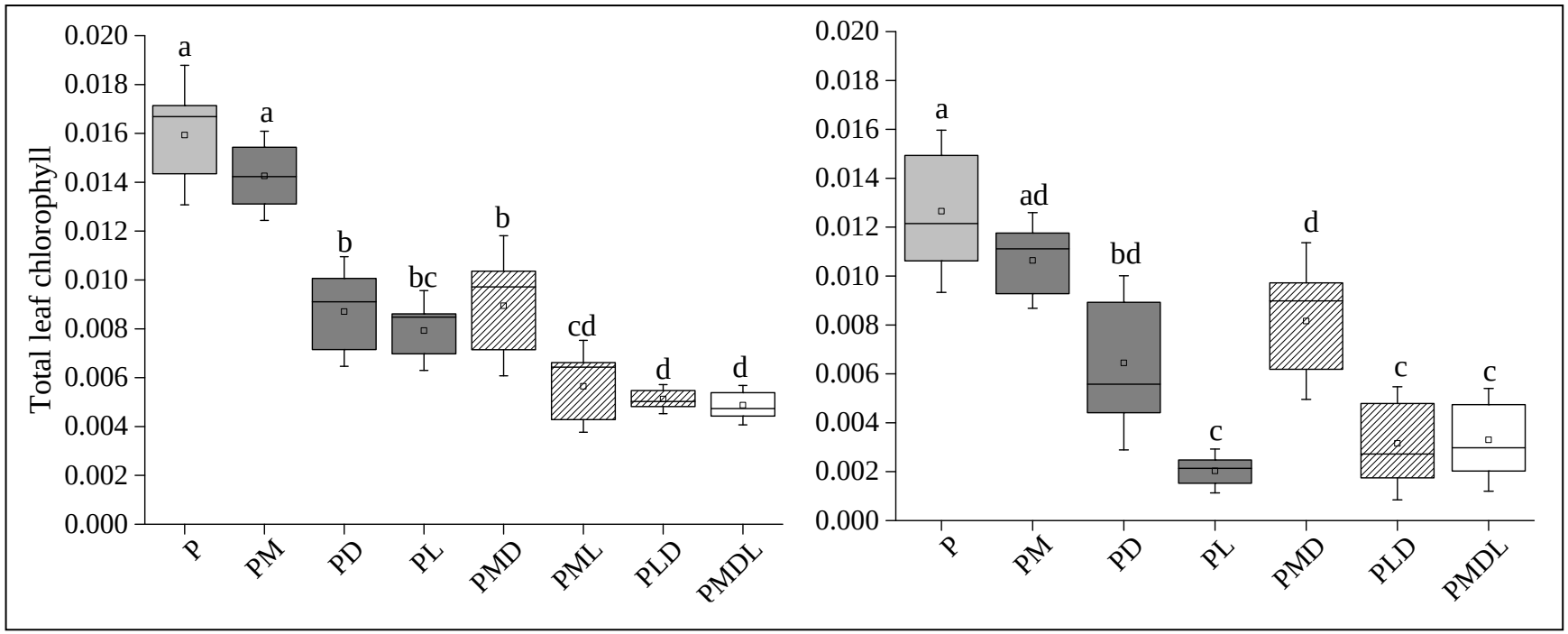


Figure 27: Mean (±SD) Total Chlorophyll Content of *P. hysterophorus* Seedlings when Grown Alone (light grey box), and with One (grey boxes), Two (dashed boxes) or three (white box) Suppressive Plant Species in Plot (left) and pot (tight) experiments. Boxes with Dissimilar Letters are Significantly Different by Tukey's HSD Test at $p \leq 0.05$. Boxplots show the Mean (square within boxes), 25% and 75% Quartile Ranges and Whiskers Show Standard Deviations. *Parthenium hysterophorus* seedlings in Planting Mixture PML in Pot Experiment Died. P = *P. hysterophorus*, M = *M. sativa*, D = *D. intortum* and L = *L. purpureus*

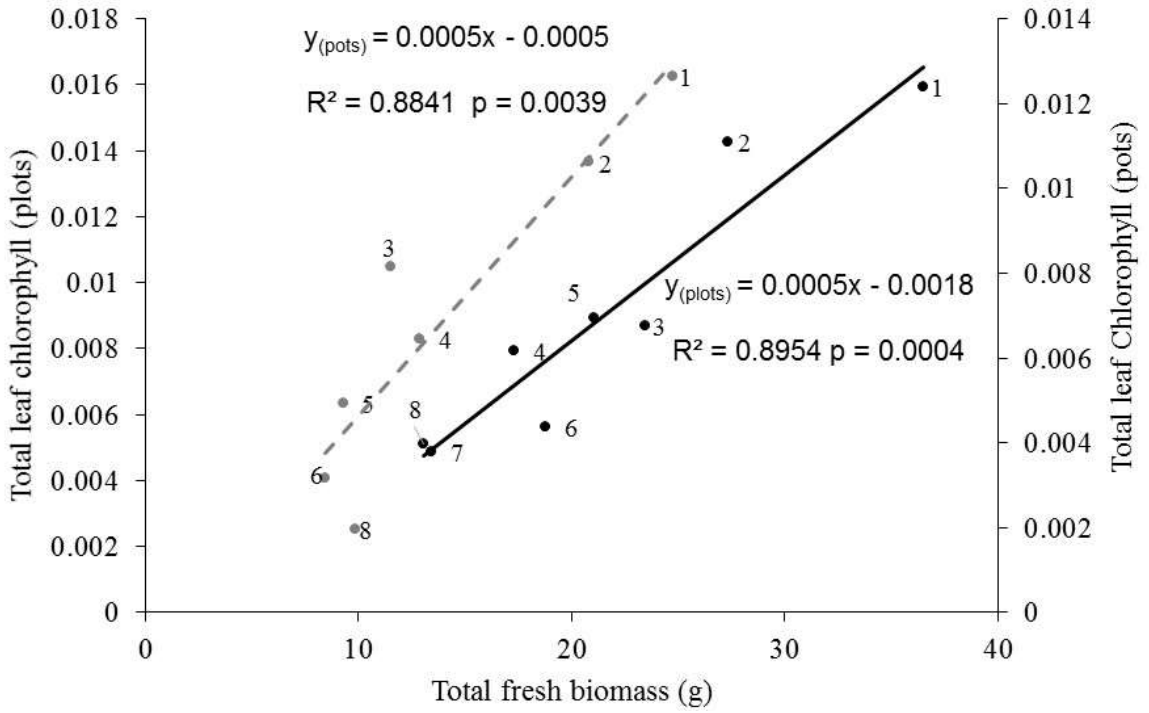


Figure 28: Relationships between Mean Total Leaf Chlorophyll Content and Total Fresh Biomass of *P. hysterophorus* Seedlings Grown in Different Planting Mixtures at Increased Suppressive Plant Species Diversity in Pot (grey dots) and Plot (black dots) Experiments

The numbers 1 to 8 represent the combination of *P. hysterophorus* with suppressive plants, 1 = P, 2 = PM, 3 = PD, 4 = PL, 4 = PMD, 6 = PLD, 7 = PML, 8 = PMDL.

4.1.8 Effects of *Desmodium uncinatum* Leaf Crude Extract on *Parthenium hysterophorus* Growth

The germination of *P. hysterophorus* seeds was delayed at higher concentrations (> 70%) of DuL crude extract compared to lower concentrations (Fig. 29a-c). Under 25% DuL concentrations and in the control treatment, seedlings had emerged at day 3 (Fig. 29a-c). Under 100% DuL concentrations, *P. hysterophorus* seed germination was suppressed by 73% in petri dishes ($F_{(4, 20)} = 13.88$, $p < 0.0001$), 60% in pots ($F_{(4, 20)} = 17.82$, $p < 0.0001$) and 57% in plots ($F_{(4, 20)} = 18.73$, $p < 0.0001$) (Table 9). In general, the seed germination inhibition increased with increasing DuL crude extract concentration treatment (Table 9).

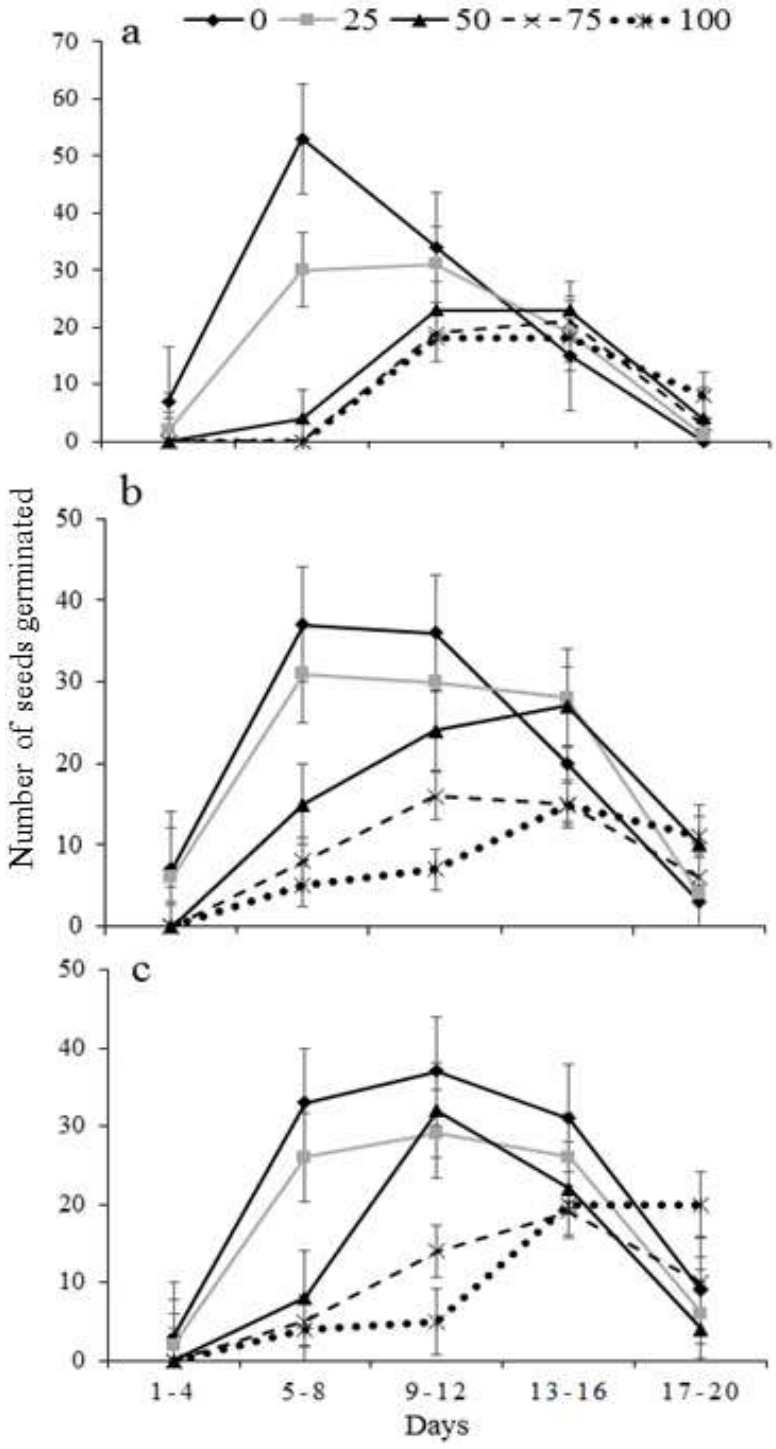


Figure 29: The Number of *P. hysterophorus* Seeds that Germinated under Different Concentration Treatments (0%, 25%, 50%, 75%, 100%) of *D. uncinatum* Leaf Crude Extract in Petri Dishes (a), Pots (b) and Plots (c) Over the Experimental Period of 20 Days

Table 9: Mean (±SE) Germination Percentage and Germination Inhibition Percentage of *P. hysterophorus* Seeds under Different Concentrations of *D. uncinatum* Crude Extract over a 20 Days Experiment in Petri Dishes, Pots and Field Plots

DuL crude extract concentrations (%)	**0**	**25**	**50**	**75**	**100**
Germination % in Petri dishes (n = 25)	86 ± 0^{a}	65 ± 2^{ab}	43 ± 2^{bc}	34 ± 3^{c}	22 ± 1^{c}
Germination % in Pots (n = 25)	77 ± 0^{a}	74 ± 1^{ab}	61 ± 2^{b}	36 ± 1^{c}	30 ± 2^{c}
Germination % in Plots (n = 25)	88 ± 0^{a}	70 ± 0^{b}	53 ± 1^{cd}	38 ± 2^{d}	39 ± 1^{d}
Seed germination inhibition percentage (%)					
Inhibition % in Petri dishes	-	21	47	58	73
Inhibition % in Pots	-	9	21	53	60
Inhibition % in Plots	-	21	40	56	57

Values with different letter (s) in a row are significantly different by Fisher LSD at $p = 0.05$

Stem height of *P. hysterophorus* seedlings sprayed with DuL concentrations differed significantly in both plot and pot experiments ($F_{(4, 20)} = 11.21$, $p = 0.0001$ and $F_{(4, 20)} = 16.87$, $p < 0.0001$ respectively, Fig. 30). Mean (± SE) stem height of *P. hysterophorus* seedlings sprayed with 75% and 100% concentrations of DuL crude extract in the plot experiment (21 ± 0.2 cm and 20.3 ± 0.5 cm respectively) was about 36% shorter than those sprayed with lower concentrations (< 70%) and control. In the pot experiment, stem height in 75% and 100% treatments (Mean ± SE: 11.3 ± 0.4 cm, 75%; and 12.1 ± 0.5 cm, 100% respectively) was approximately 30% shorter than that sprayed with lower DuL concentrations (<50%). The root length of *P. hysterophorus* seedlings sprayed with DuL crude extract concentrations differed significantly in both plot and pot experiments ($F_{(4, 20)} = 27.80$, $p < 0.0001$ and $F_{(4, 20)} = 3.83$, $p = 0.0181$ respectively, Fig. 31).

The root length of seedlings in 50%, 75% and 100% concentrations of DuL in plot experiment were about 45% shorter than those sprayed with 25% of DuL concentration and control. In pot experiments, the root length was about 51% shorter in plants sprayed with 75% or higher concentrations than those sprayed with 25% and 50% concentrations (Mean ± SE: 7.7 ± 0.6 cm, 75%; 8.8 ± 0.6 cm, 100%).

The stem or shoot diameter of *P. hysterophorus* seedlings differed significantly under different DuL concentrations in both plot and pot experiments ($F_{(4, 20)} = 3.19$, $p = 0.0351$, and $F_{(4, 20)} = 12.26$, $p < 0.0001$ respectively, Fig. 32). The shoot diameter of seedlings sprayed with 50%, 75% and 100% concentrations of DuL in plot experiments was slightly smaller than those sprayed with 25% concentration of DuL. In the pot experiments the shoot diameter

was approximately 31% smaller than those sprayed with 25% and 50% concentrations (Mean ± SE: 2.4 ± 0.1 mm, 75% and 2.2 ± 0.2 mm, 100%).

Average AFB of *P. hysterophorus* seedlings differed significantly with DuL crude extract concentrations in both field plot ($F_{(4, 20)}$ = 3.31, p = 0.031) and pot ($F_{(4, 20)}$ = 16.16, p < 0.0001) experiments (Fig. 33). Also, the BFB in field plots and pots experiments was significantly different between treatments ($F_{(4, 20)}$ = 51.85, p = 0.031 and $F_{(4, 20)}$ = 15.95, p < 0.0001 respectively, Fig. 34). In both plot and pot experiments, the seedlings sprayed with 100% concentration of DuL crude extract were observed to have lower AFB (Fig. 33) and BFB (Fig. 34). Respectively, the AFB in plots and pots (Mean ± SE: 110.1 ± 6.1 g, plots, and 10.7 ± 0.9 g, pots) was about 33% and 30% smaller than AFB in lower concentrations. Similarly, the BFB (Mean ± SE: 6.2 ± 0.3 g, plots and 2.1 ± 0.3 g, pots) was about 60% and 78% smaller than BFB in lower concentrations in plots and pots experiments respectively.

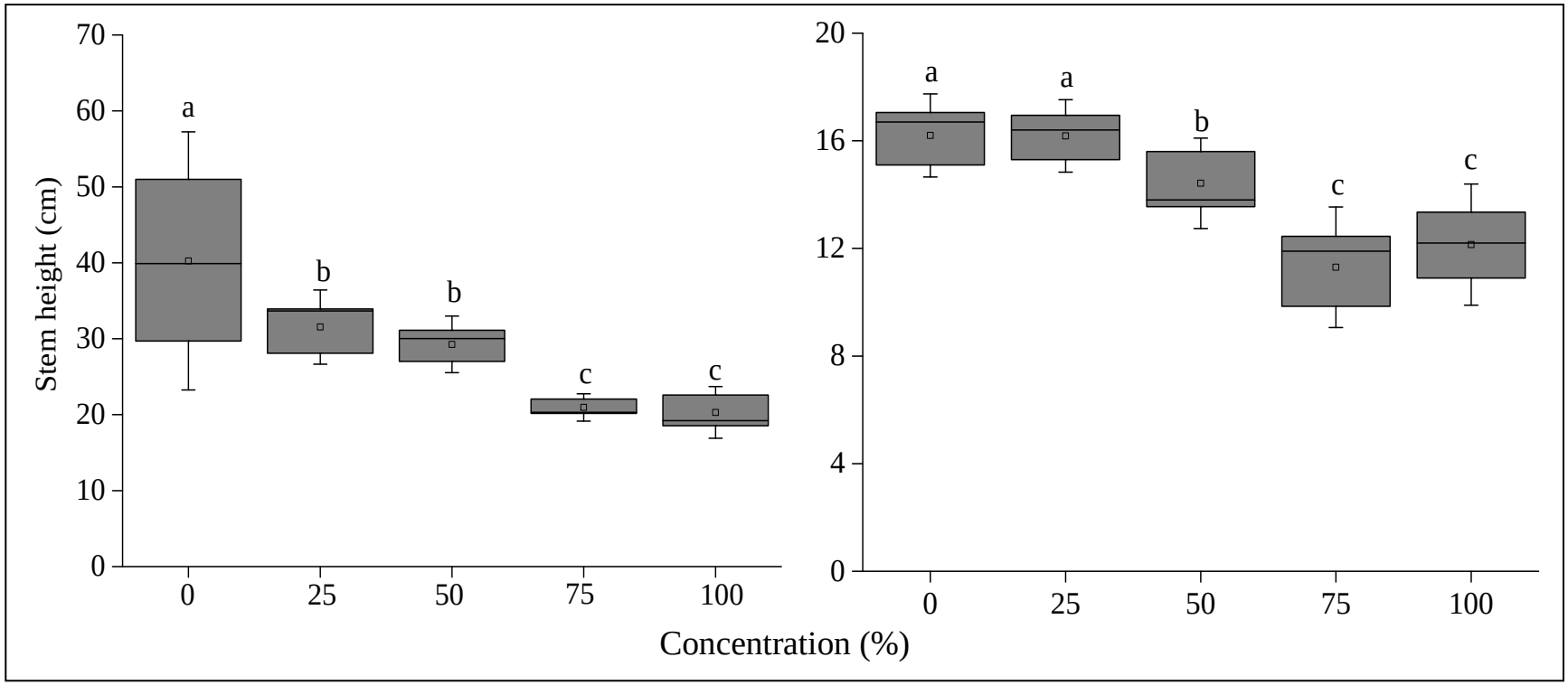


Figure 30: Stem height of *P. hysterophorus* Seedlings Treated with *D. uncinatum* Leaf Crude Extract for 25 Days in Field Plots (left) and pots (right) Experiments under Different Concentration. Boxes with Different Letter(s) are Significantly Different by Fisher LSD at p = 0.05. Boxplots Show the Mean (square within boxes), 25% and 75% Quartile Ranges and Whiskers Show Standard Deviations

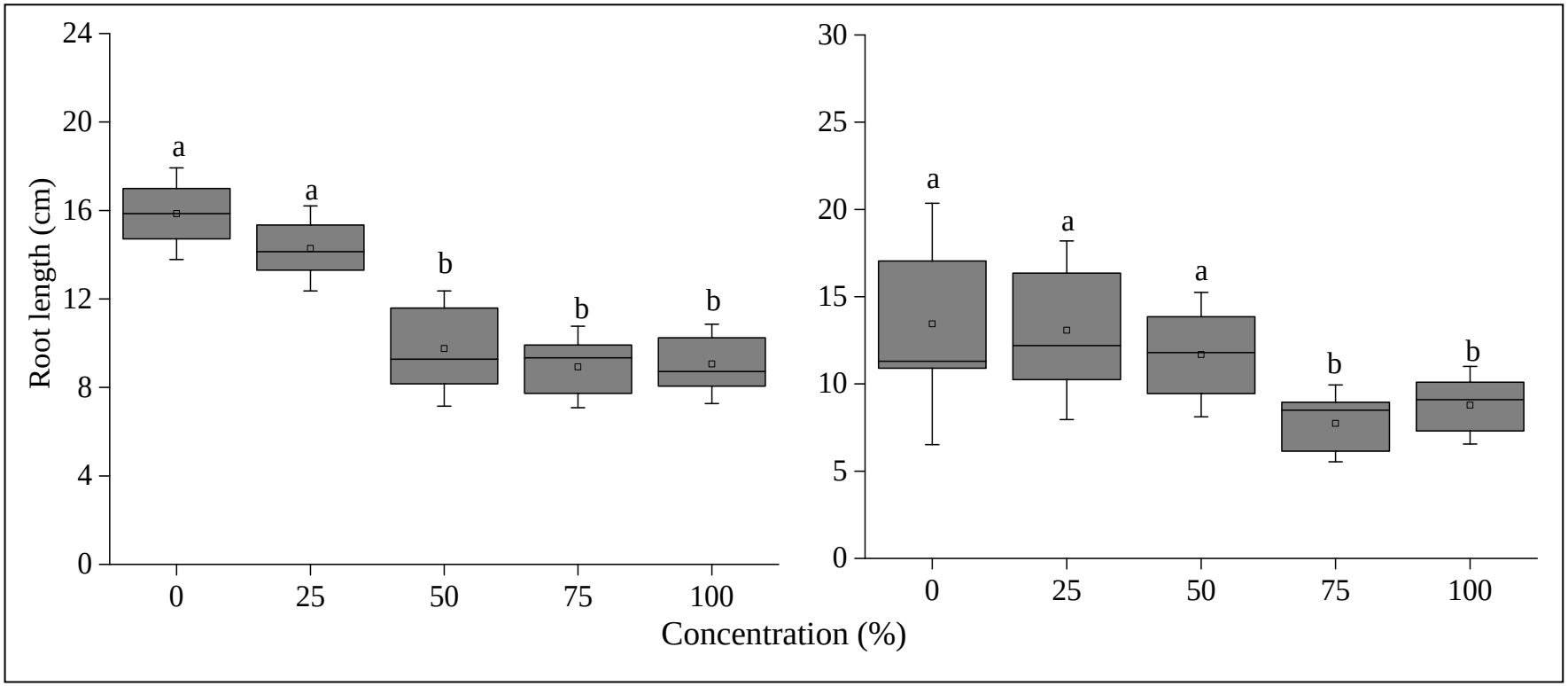


Figure 31: Root Length of *P. hysterophorus* Seedlings Treated with *D. uncinatum* Leaf Crude Extract for 25 Days in Field Plots (left panels) and Pots (right panels) Experiments under Different Concentration. Boxes with Different Letter(s) are Significantly Different by Fisher LSD at p = 0.05. Boxplots Show the Mean (square within boxes), 25% and 75% Quartile Ranges and Whiskers Show Standard Deviations

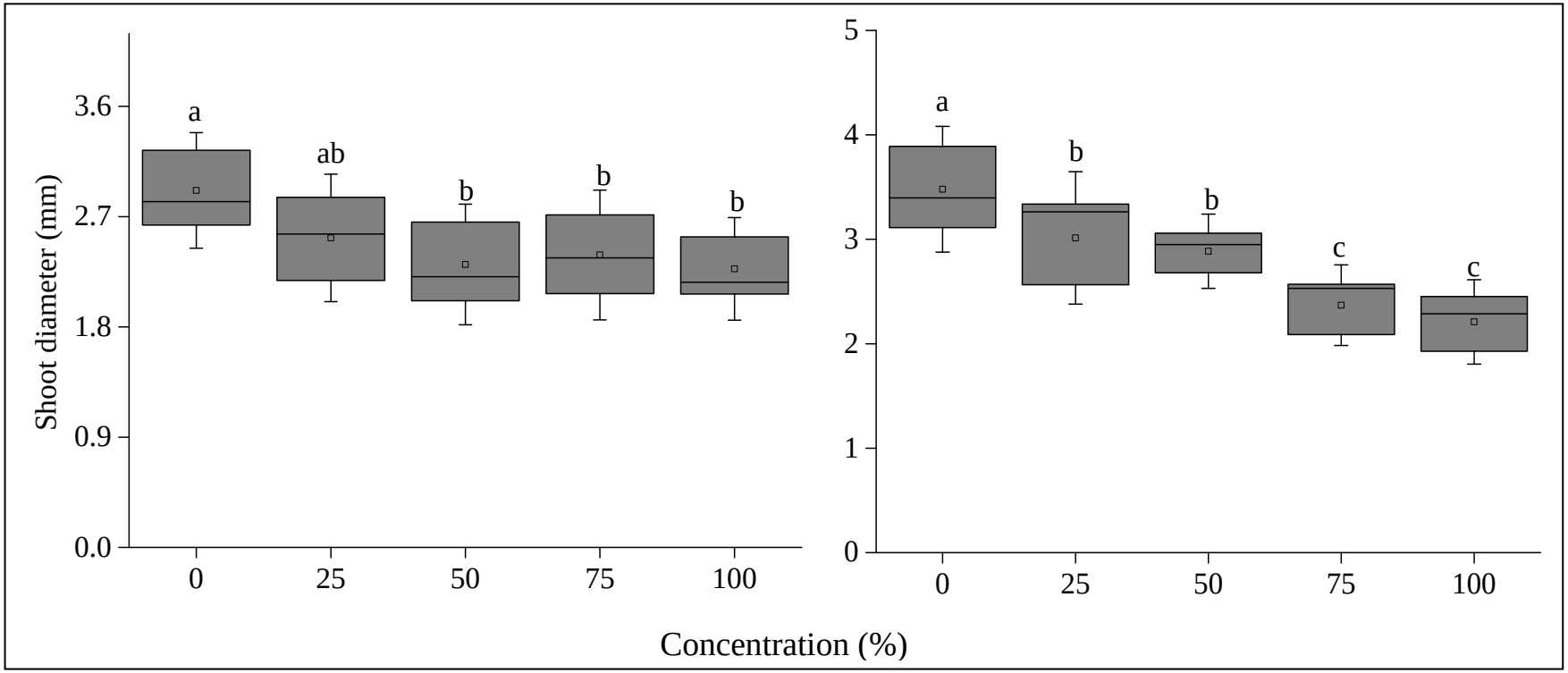


Figure 32: Shoot Diameter of *P. hysterophorus* Seedlings Treated with *D. uncinatum* Leaf Crude Extract for 25 Days in Field Plots (left panels) and Pots (right panels) Experiments under Different Concentration. Boxes with Different Letter(s) are Significantly Different by Fisher LSD at p = 0.05. Boxplots Show the Mean (square within boxes), 25% and 75% Quartile Ranges and Whiskers Show Standard Deviations

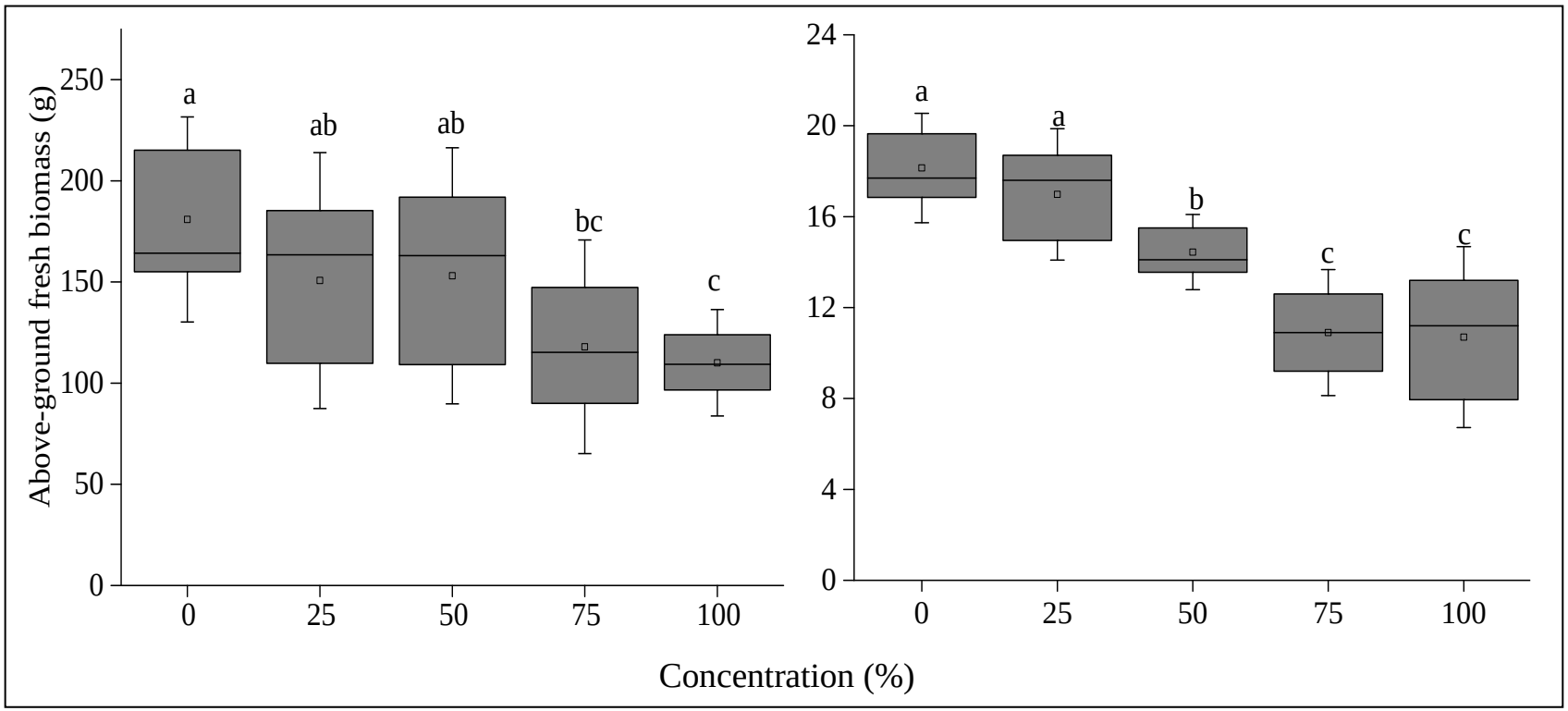


Figure 33: Above–Ground Fresh Biomass of *P. hysterophorus* Seedlings Sprayed with *D. uncinatum* Leaf Crude Extract (DuL) after 25 Days in Field Plots (left) and Pots (right) Experiments under Different DuL Concentration. Boxes with Different Letter(s) are Significantly Different by Fisher LSD at $p = 0.05$. Boxplots Show the Mean (square within boxes), 25% and 75% Quartile Ranges and Whiskers Show Standard Deviations

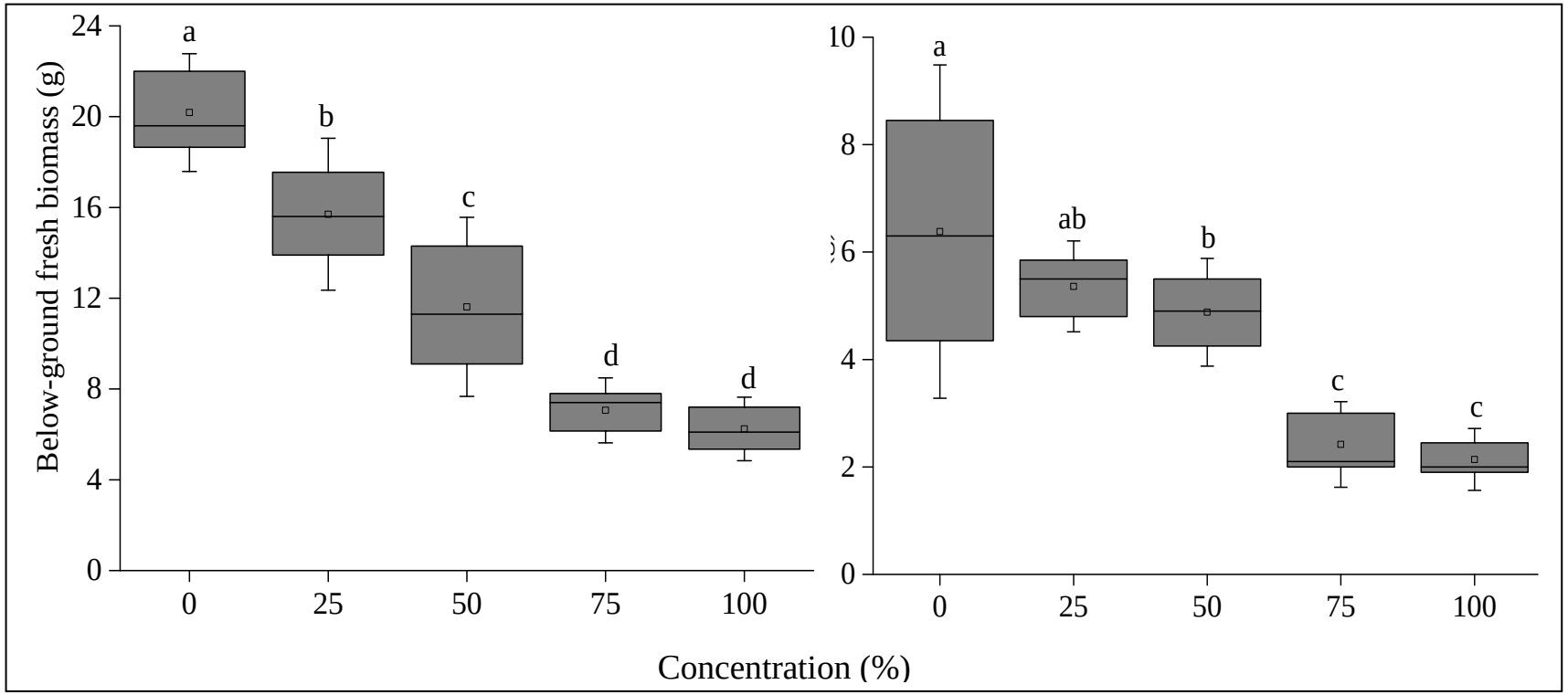


Figure 34: Below–Ground Fresh Biomass of *P. hysterophorus* Seedlings Sprayed with *D. uncinatum* Leaf Crude Extract (DuL) after 25 Days in Field Plots (left) and Pots (right) Experiments under Different DuL Concentration. Boxes with Different Letter(s) are Significantly Different by Fisher LSD at $p = 0.05$. Boxplots Show the Mean (square within boxes), 25% and 75% Quartile Ranges and Whiskers Show Standard Deviations

Average ADB of *P. hysterophorus* seedlings differed significantly under DuL crude extract concentrations in both field plots ($F_{(4, 20)}$ = 6.30, p = 0.0019) and pots ($F_{(4, 20)}$ = 42.39, p < 0.0001) (Fig. 35). Similarly, BDB in field plots and pots experiments was significant different ($F_{(4, 20)}$ = 5.14, p = 0.0052, and $F_{(4, 20)}$ = 13.19, p < 0.0001 respectively, Fig. 36). The seedlings sprayed with 100% concentration of DuL crude extract were observed to have lower ADB (Fig. 34) and BDB (Fig. 35) in both experiments. Respectively, the ADB in plots and pots (Mean ± SE: 10.1 ± 0.8 g, plots, and 2.1± 0.1 g, pots) was 41% and 50% smaller than the ADB in lower concentrations. The BDB of seedling under high DuL concentration treatments in pots (Mean ± SE: 0.7 ± 0.5 g) was about 67% smaller than that in lower concentration (Fig. 35). With 75% and 100% DuL concentrations, the BDB in plots was considerably reduced (i.e. > 75%) compared to BDB in lower concentrations (Mean ± SE: 0.7 ± 0.0 g).

4.1.9 Effects of *D. uncinatum* Leaf Crude Extract on *P. hysterophorus* Leaf Chlorophyll Content

Total leaf chlorophyll content of *P. hysterophorus* seedlings differed significantly between concentrations of DuL crude extract in both plots and pots experiments ($F_{(4, 20)}$ = 54.96, p < 0.0001 and $F_{(4, 20)}$ = 6.86, p = 0.0012 respectively, Fig. 37). Seedlings sprayed with higher DuL concentrations (75% and 100%) had lower total Chl content (i.e. < 22% and 26% in plots and pots respectively) than those sprayed with lower concentrations.

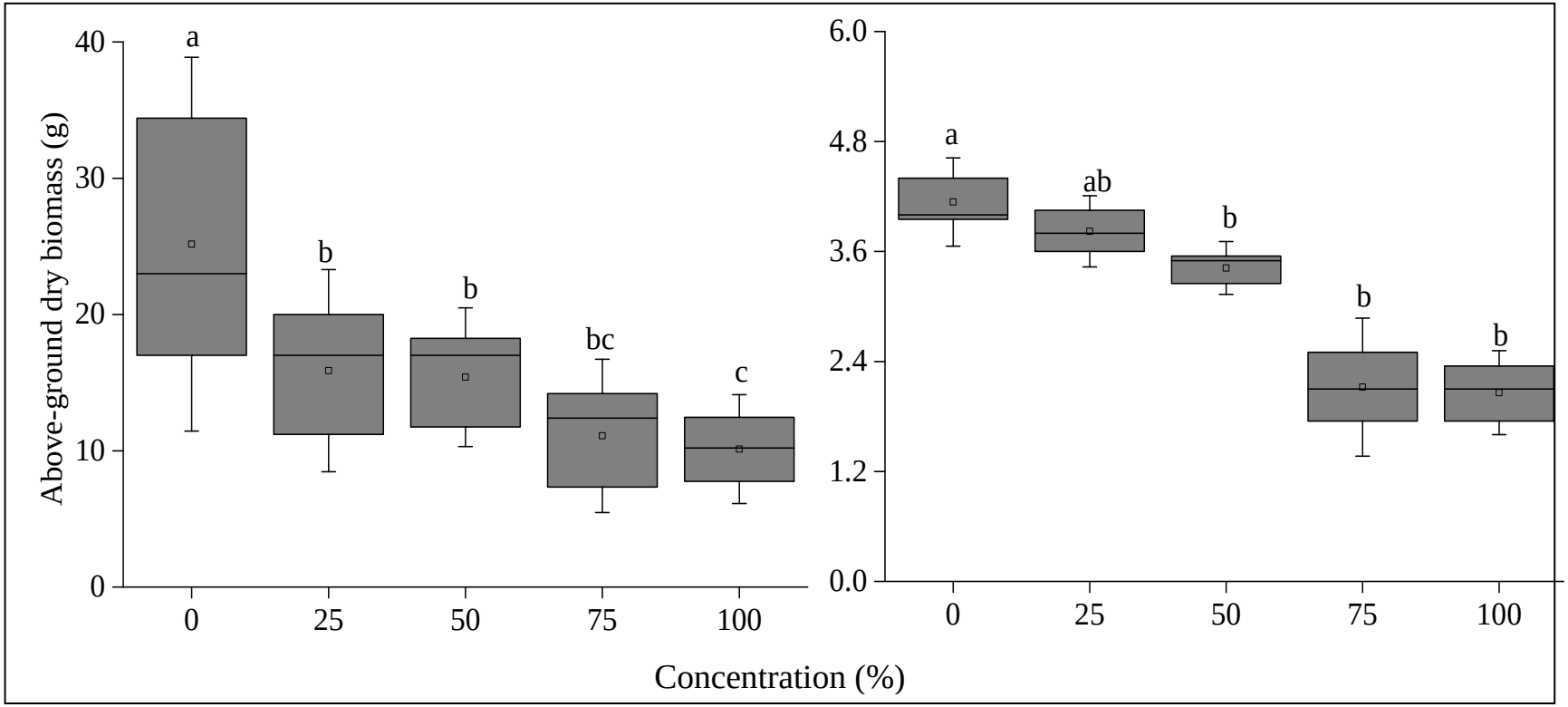


Figure 35: Above–Ground Dry Biomass of *P. hysterophorus* Seedlings Sprayed with *D. uncinatum* Leaf Crude Extract for 25 Days in Field Plots (left panels) and Pots (right panels) Experiments under Different Concentration. Boxes with Different Letter(s) are Significantly Different by Fisher LSD at $p = 0.05$. Boxplots Show the Mean (square within boxes), 25% and 75% Quartile Ranges and Whiskers Show Standard Deviations

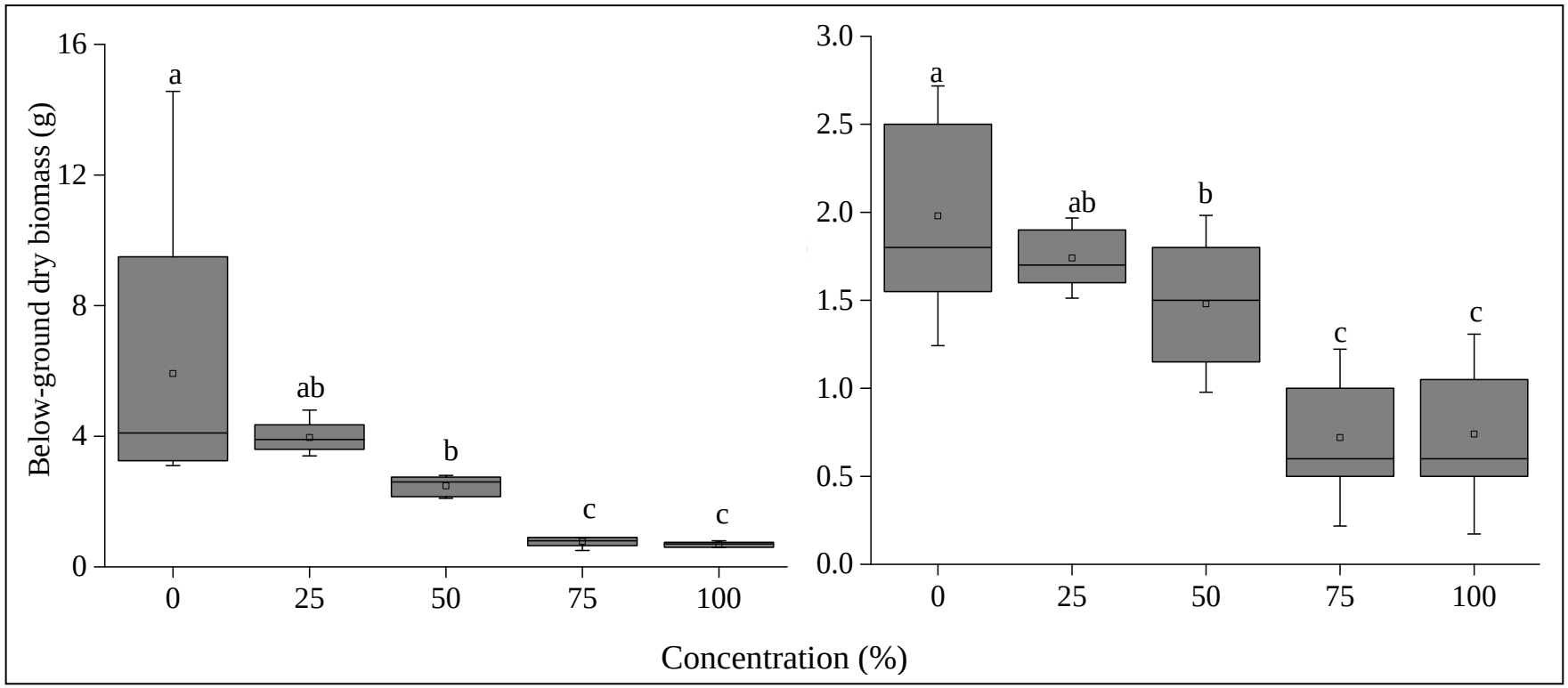


Figure 36: Below–Ground Dry Biomass of *P. hysterophorus* Seedlings Sprayed with *D. Uncinatum* Leaf Crude Extract for 25 Days in Field Plots (left) and Pots (right) Experiments under Different Concentration. Boxes with Different Letter(s) are Significantly Different by Fisher LSD at $p = 0.05$. Boxplots Show the Mean (square within boxes), 25% and 75% Quartile Ranges and Whiskers Show Standard Deviations

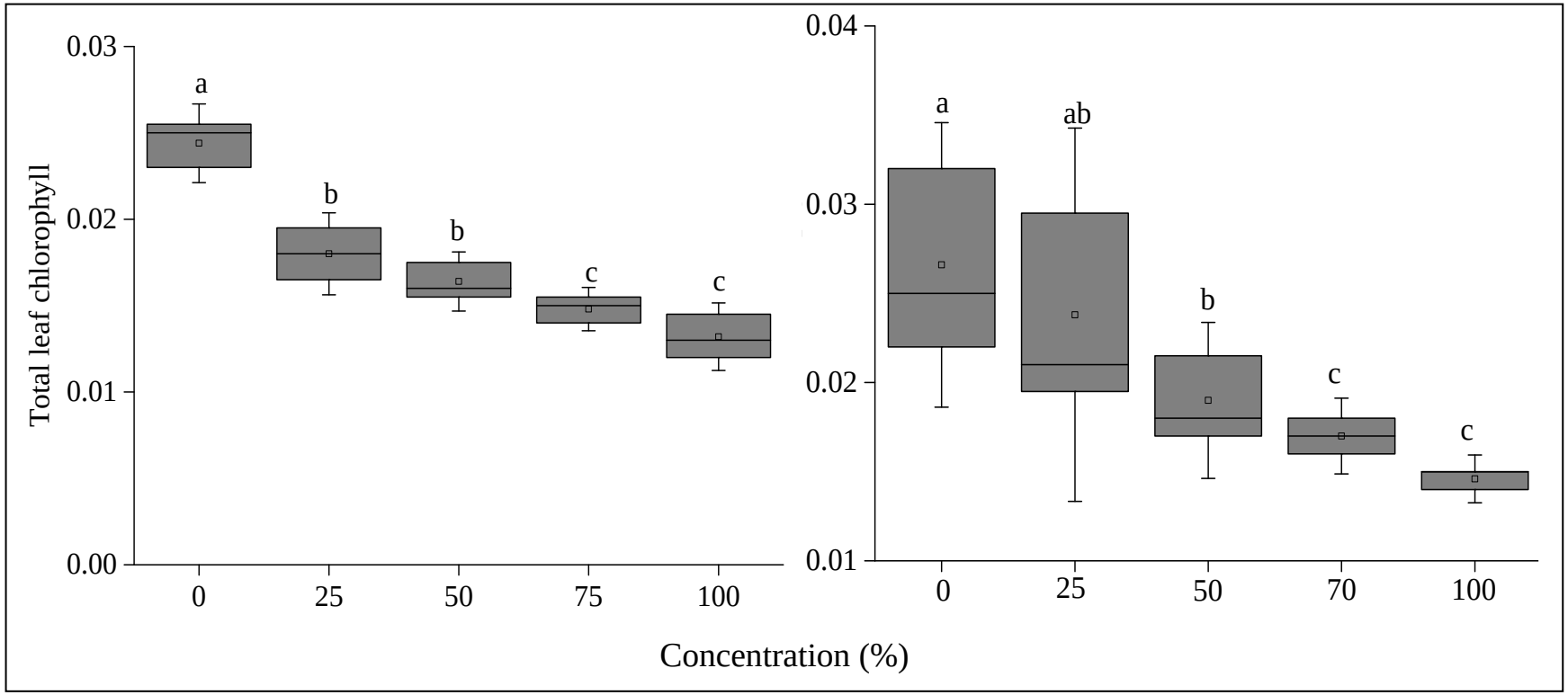


Figure 37: Total Leaf Chlorophyll Content of *P. hysterophorus* Seedling Sprayed with *D. uncinatum* Leaf Crude Extract for 25 days in Field plots (left) and Pots (right) and Experiments under Different Concentration. Boxes with Different Letter(s) are Significantly Different by Fisher LSD at $p = 0.05$. Boxplots Show the Mean (square within boxes), 25% and 75% Quartile Ranges and Whiskers Show Standard Deviation

4.2 Discussion

4.2.1 *Parthenium hysterophorus* Distribution Within and Outside Arusha National Park and its Impact on Soil Chemical Properties

This study revealed that *P. hysterophorus* has invaded some villages (settlements, maize and banana fields) neighbouring the ANP. Although the park is currently uninvaded, *P. hysterophorus* invasion in the adjacent villages and along the Momela road which enters the ANP shows that vehicular transportation can be the major way of dispersal into the park. The invasion in the villages might be due to human activities such as grazing and fodder collection, as well as vehicles which disperse *P. hysterophorus* seeds. This current invasion at the border zones put the park's biodiversity and ecology under risk. Villagers' socio–economic activities nearby the ANP border zones can easily promote dispersal of *P. hysterophorus* seeds into the park. Besides, *P. hysterophorus* seeds can be transported into the park as a contaminant of tourists and staffs in their belongings or carry the seeds in mud adhered to their shoes (Gervilla *et al*., 2019). Vehicles from Arusha and other neighbouring areas invaded with *P. hysterophorus* may also transport the invasive seeds in mud adhered to tyres into the park and/or its border zones. Since *P. hysterophorus* invasions follow disturbances, any form of activities leading to environmental disturbances should be prohibited at the ANP border zones. As the roadsides are preferential migration corridor and the starting points of *P. hysterophorus* invasions into adjacent surroundings (Christen & Matlack, 2006; Johnston & Johnston, 2004; Von Der Lippe & Kowarik, 2007; Wabuyele *et al*., 2015), the invaded Momela road increases the chance of spreading its seeds into the ANP.

The frequency of *P. hysterophorus* occurrence was high in maize field compared to banana and grazing fields possibly due to the fact that maize fields are tilled more often than others. Tillage has been reported to facilitate the spread of invasives by fragmenting and transporting reproductive structures (i.e. rhizomes) and seeds (DiTomaso *et al*., 2010). Similarly, it creates disturbed areas that are rapidly occupied by invasive plants such as *P. hysterophorus* (DiTomaso *et al*., 2010). This is the reason why it is not practiced usually on rangeland (DiTomaso *et al*., 2010). In this view, frequent tillage may have facilitated more *P. hysterophorus* invasion in maize fields. Furthermore, Vera (1997) and Gale (2004) described that high elevation limit or affect seed germination as well as growth characteristics of some plant species either due to limited temperature, high ultraviolet **(**UV) radiation, excessive

rainfall or competition with other adapted plants at higher altitude. Nevertheless, in this study it is unsure if these factors also limited *P. hysterophorus* growth, and so its invasion in ANP. However, it is suspected that *P. hysterophorus* seed being very small in size and light are easily washed by running water along the slope to lower elevation before reaching in the park. This might be a reason for the ANP which is located at higher elevation not being invaded currently by *P. hysterophorus*. A study in Arumeru area of Arusha also found that most areas affected by *P. hysterophorus* ranged in elevation >1000 m.a.s.l (Wabuyele *et al*., 2015). This agrees with the findings of this study that *P. hysterophorus* invasion was recorded mostly at lower elevations in villages. Similar results by Etana *et al*. (2015) suggest that *P. hysterophorus* invasions in Awash National Park in Ethiopia decreased with increasing altitude. Despite that high elevation may delay *P. hysterophorus* invasion in ANP, environmental factors can favour its invasions into ANP and other protected areas in Tanzania as the invasive grows virtually on all types of soils and habitats except near the seashore.

Thus, in order to prevent accidental introduction of *P. hysterophorus* into ANP, vehicular traffic in the park should be limited, and livestock movements near the park border zones must be banned. Similarly, construction of roads and lodges in the park should not use construction materials such as soil from areas invaded with *P. hysterophorus*. Further, the study findings indicate that *P. hysterophorus* may be associated with certain soil chemical properties such as N, P, Ca, Mg, K, OC and Mn, as was also found by Etana *et al*. (2015) and Osunkoya *et al*. (2017). Most soil chemical properties from quadrats invaded with *P. hysterophorus* in this study did not differ significantly from uninvaded quadrats. Nevertheless, it was found that higher soil phosphorus in quadrats not invaded with *P. hysterophorus*, unlike the study of Timsina *et al*. (2011) in Nepal. This discrepancy might be due to the nature of soil, different vegetation growing in the two areas, collection, storage, and preparation of soil samples in the two countries. Further, high value of CEC in invaded site with decreasing organic matter, Ca, Mg and K in the invaded site could be due to increasing acidic cations i.e. H^+ or Mn^{2+} in the soils. As soils become more acidic Ca, Mg and K are replaced by H+ or Mn^{2+} and this may result in CEC values much higher than expected (McKenzie *et al*., 2004). In general, this study shows that *P. hysterophorus* is continuing to spread and may eventually invade protected areas in Tanzania. It may also alter soil–chemical properties by utilizing available nutrients.

4.2.2 Impact of *P. hysterophorus* on Flower Visitation to Co–Flowering Plants

It was found that *P. hysterophorus* receives visits from different insect flower visitors such as *Apis mellifera*, Calliphoridae, Coccinellidae, Syrphidae, *B. aurota*, Melyridae, Meloidae and Hemiptera. *Parthenium hysterophorus* ray florets (female part) rich in nectar and pollen may be attracted flower visitors and enhanced the visitors' foraging activity on invasive flowers (Kaur *et al*., 2014; Usharani & Raju, 2018). Thus, the presence of *P. hysterophorus* increased the number of potential pollinators, specially *A. mellifera*, in the invaded quadrats in this study. Nevertheless, its presence attracted these flower visitors away from target plant species (*O. gratissimum* and *A. conyzoides*), which in the case of native plants implies the potential for a strong negative effect on wild plant reproductive success.

Previous studies showed that flower visitors or potential pollinators to flowering plants are attracted by floral abundance (Ghazoul, 2004; Lopezaraiza–Mikel *et al*., 2007) and floral morphology (McKinney & Goodell, 2011). Generalised flowers with easy access to the nectar tend to attract a larger diversity of non–specialist visitors compared to flowers with specialised morphology such as long corollas or complex mechanisms (Johnson & Steiner, 2000). Common to many Asteraceae, the non–tubular flowers of *P. hysterophorus* (Kaur *et al*., 2014; Usharani & Raju, 2018) likely attract predominantly generalist flower visitors such as *A. mellifera* and Syrphidae (hoverflies). These generalists visited other wild plants in the study area less frequently in the presence of *P. hysterophorus*, which agrees with other previous studies on IAPs (Brown *et al*., 2002; Jakobsson *et al*., 2008; Sun *et al*., 2013; Totland *et al*., 2006). The results are consistent with that of Stiers *et al*. (2014) which showed that invasive *Ludwigia grandiflora*, a plant with similar generalised floral morphology and accessible nectar/pollen, can reduce the number of arriving pollinators and visitation rate of native *Lythrum salicaria*. Gibson *et al*. (2013), Lopezaraiza–Mikel *et al*. (2007) and Morales and Traveset (2008) also found that presence of the invasive *Phacelia tanacetifolia* had strong negative effects on the visitation rate to the native *Melampyrum pratense*. A high visitation rate from pollinators enhances gene flow within plant populations and contributes to natural plants community stability (Gibson *et al*., 2013; Lopezaraiza–Mikel *et al*., 2007; Morales & Traveset, 2008).

The findings that *P. hysterophorus* lowered flower visitor frequencies and visitation rates to wild plants suggest that *P. hysterophorus* has the potential to disrupt native plant–pollinator networks, which can have wider–reaching impacts on abundance and diversity of native

plants (Gibson *et al*., 2013; Lopezaraiza–Mikel *et al*., 2007; Morales & Traveset, 2008). Its invasions may interrupt pollen flow between native plants in invaded ecosystems and harm their reproduction by reducing seed set (Albano *et al*., 2009; Chittka & Schürkens, 2001; Sun *et al*., 2013). In the visitation networks *P. hysterophorus* interacted with native and introduced co–flowering plants by sharing a wide range of insect flower visitors like other alien invasive plants (Aizen *et al*., 2008; Albrecht *et al*., 2014; Bartomeus *et al*., 2008; Padrón *et al*., 2009). Some of these visitors are pollen vectors such as *A. mellifera* and Syrphidae. *Apis mellifera* played a significant role as an integrator of *P. hysterophorus* into flower visitor networks in this study (Barrios *et al*., 2016; Stiers *et al*., 2014). This generalist flower visitor tended to visit diverse flowers of different plant species including *P. hysterophorus*. However, *A. mellifera* individuals show one of the highest levels of floral constancy of any pollinator (Chittka *et al*., 1999).

High nestedness was found in the uninvaded sites which indicates the presence of more interactions and generalist dominance (Blüthgen *et al*., 2008), and higher stability of networks in these sites (Dormann *et al*., 2009). As *P. hysterophorus* is a generalist plant (species with many links) which receives both generalist and specialist flower visitors, it could push out specialist plants (species with few links) from the networks by attracting flower visitors away from these plants (Blüthgen *et al*., 2008). Low linkage density in invaded sites infers that *P. hysterophorus* decreases plant–insect flower visitor interactions in these sites (Ferrero *et al*., 2013; Padrón *et al*., 2009). Invasive species other than *P. hysterophorus* also have shown significant consequences for the plant–pollinator network structure. For instance, the invasive species *Carpobrotus affine acinaciformis* and *Opuntia stricta* appeared to alter plant–pollinator structure, whereby the former species competed with native species for pollinators and so increased nestedness and the later facilitated pollinator visits to native plant species (Bartomeus *et al*., 2008). Integration of *P. hysterophorus* into networks reduced native plant–pollinator interactions and therefore lead to reduced robustness. The consequences of this could include the disruption of pollination networks, reduced native plant seed set productivity and community stability. But, in the field sites, *P. hysterophorus* did not show a large effect on the visitation network structure. Since *P. hysterophorus* is facultatively autogamous and anemophilous, it will set seed even without the presence of insect flower visitors (Martins, 2014; Usharani & Raju, 2018). However, it may be drawing pollinators away from plants that need them more than this invasive species. Native plants reliant on pollinators to transport pollen between individuals are more prone to

competition for pollination with *P. hysterophorus* than self–compatible plants able to carry out autonomous pollination (Jakobsson *et al*., 2008; Nielsen *et al*., 2008). Hence, other plant species, in contrast, will not be able to survive without pollinators. As visitation rate and number of flower visitors to native co–flowering plants are negatively affected in the presence of *P. hysterophorus*, thus, the invasive must be controlled to ameliorate negative impacts on native and established plant communities (Fantinato *et al*., 2018).

Given the allelopathic effects of this species, it is possible that the pollen of *P. hysterophorus* may have traits to which co–flowering plants are not adapted and may hinder fertilization due to stigma–clogging in native flowers (Albano *et al*., 2009; Chittka & Schürkens, 2001; Flanagan *et al*., 2009; Kaiser-Bunbury & Müller, 2009; Nielsen *et al*., 2008). Such effects have been reported in other invasive species including *Carpobrotus* spp, *Oxalis pes-caprae*, *Lythrum salicaria* and *Heracleum mantegazzianum* (Jakobsson *et al*., 2008; Nielsen *et al*., 2008). Moreover, the shorter duration of visits by *A. mellifera*, Syrphidae, other Diptera and some Lepidoptera to flowers of *O. gratissimum* and *A. conyzoides* in invaded quadrats could be due to competition with *P. hysterophorus*. As many Coleoptera (Shimamura *et al*., 2005) and Diptera (Irvin *et al*., 1999) are pollen feeders, it is anticipated that these taxa were attracted to flowers of *P. hysterophorus* due to volume of pollen in this study. Further, in this study, the visitation rate of Diptera to flowers of *A. conyzoides* was not strongly affected by *P. hysterophorus* which could be due to their similar flower morphology or a stronger preference for *A. conyzoides* among this taxon, perhaps because of cues such as colour or odour.

Overall, these findings suggest that *P. hysterophorus* has potential to displace native plants via competition for pollinator visits as it exerted a magnet species effect on *A. mellifera* and Syrphidae (Gibson *et al*., 2013; Molina-Montenegro *et al*., 2008). By attracting flower visitors that could otherwise serve as pollinators of native species and crops, *P. hysterophorus*, which is rapidly spreading in Africa could have complex harmful effects on the wider ecosystem. As a relatively high number of generalist pollinators visit *P. hysterophorus*, it is expected to see high seed sets in its existing habitats and the potential to invade more areas. As it continues to spread in crop fields and natural habitats could reduce pollination to native flowering plants and crops, thereby threatening biodiversity and farmers' livelihoods in Tanzania. Therefore, *P. hysterophorus*, which was previously known to exert

competitive effects on native plants via allelopathy, has been shown to do so also through competition for flower visitors.

4.2.3 Effects of Selected Suppressive Plant Species on *P. hysterophorus* Growth Vigour

A management trial using suppressive forage plant species revealed that *P. hysterophorus* growth was negatively affected when it was grown with the suppressive species in mixtures, which is in agreement with Khan *et al*. (2013), Shabbir *et al*. (2013) and Zheng *et al*. (2015). *Lablab purpureus* was the primary species responsible for suppression of *P. hysterophorus* growth, as all performance parameters were constantly low across planting mixtures with *L. purpureus*. In mixtures that did not contain this species, little or no significant suppressive effect was observed. When the numbers of *D. intortum*, *L. purpureus* and *M. sativa* in the pot or field plot was increased from one to two or three suppressive species, *P. hysterophorus* stem height, shoot diameter, root length, biomass and leaf chlorophyll content decreased accordingly. This decrease followed a gradient of effectiveness i.e. the most effective plant species were *L. purpureus* > *D. intortum* > *M. sativa,* with little evidence that *M. sativa* alone could exert a suppressive effect. However, it is suggested that both the more and less suppressive species can be used together as rehabilitative species to complement each other in suppressing *P. hysterophorus* and improving livestock or wildlife forage availability, and increasing ecosystem resilience against *P. hysterophorus* invasions (Christina *et al*., 2015; Cummings *et al*., 2012). The study findings suggest that high plant density in grasslands may reduce ecosystem invasibility (Knops *et al*., 1999) and highlights the importance of keeping rangelands from becoming dominated by few grazing–tolerant species (Connolly *et al*., 2018).

Competition intensity indices (RCI > 0 and RII < 0) revealed that suppressive plants at higher species numbers negatively affected *P. hysterophorus* total fresh biomass, as it was found for other invasive species (Grace, 1995; Vilà *et al*., 2004; Weigelt & Jolliffe, 2003). Khan *et al*. (2013) found that *C. ciliaris, Setaria incrassata*, *Panicum maxicum* and *Eulalia aurea* at higher abundance suppressed *P. hysterophorus* and Ammondt and Litton (2012) showed that the invasive grass *Megathyrsus maximus* stem heights and biomass were reduced when planted together with competitive species *Myoporum sandwicense, Dodonaea viscosa*, and *Plumbago zeylanica*. The study results support that of Vilà and Weiner (2004) who reported that resistance imposed by a single native plant species to invasive species is week compared to when several native species are present. This advocates that competitive plants seeded with

P. hysterophorus in mixture of high species density may suppress the invasive species, which is in accordance with studies that found high plant density suppressed invasive abundance in pastures (Connolly *et al*., 2018; Khan *et al*., 2013; Shabbir *et al*., 2013; Tracy *et al*., 2004).

Since ecosystem invasibility is influenced by available resources in the habitat (Perkins *et al*., 2011; Tracy *et al*., 2004), increasing density or diversity of effective suppressive forage species may reduce ecosystem vulnerability to *P. hysterophorus* invasion as there is a complete resource utilization (Connolly *et al*., 2018; Knops *et al*., 1999). Thus, this study suggest that high plant diversity in grassland may reduce ecosystem invasibility and highlights the importance of keeping rangelands from becoming impoverished (Connolly *et al*., 2018; Knops *et al*., 1999). Similarly, maintaining diverse forage plant communities may affect the amount of IAPs' seed bank in the soil (Tracy *et al*., 2004). As *P. hysterophorus* seeds have a long dormancy (Brunel *et al*., 2014; Timsina *et al*., 2011), management techniques such as keeping high native forage species density or diversity might help to decrease the accumulation of the invasive seeds in soil and avoid future invasions.

Moreover, in the competition experiments it was observed that the large ground cover of *L. purpureus* shaded the rosettes of *P. hysterophorus* and likely reduced their growth due to their structural features, similar to findings of Tamado *et al.* (2002) and Khan *et al.* (2013). This observation highlight that management approaches to mitigate *P. hysterophorus* using suppressive forage plant species should target its rosettes. While *D. intortum* has been recommended for conservation as ground cover and pasture (Kariuki *et al*., 1999; Maina *et al*., 2006), this study has shown that it can also be used to control *P. hysterophorus*, particularly when mixed with the most effective suppressive legume fodder plants or grass species such as *L. purpureus*, *Digitaria eriantha*, *Urochloa mutica* and *Pennisetum clandestinum* as it likely grows better in mixed stands (Aganga & Tshwenyane, 2003). In addition to its extensive ground cover, *L. purpureus* also exhibits high stem height, root length and biomass.

Drought tolerance and nitrogen fixing traits of selected suppressive species possibly enhanced their competitive fitness in mixture over *P. hysterophorus* (Amole *et al*., 2013; Latrach *et al*., 2014; Lei *et al*., 2018; Madzonga & Mogotsi, 2014; Maina *et al*., 2006; Radovic *et al*., 2009). But, none of the three suppressive species facilitated the growth of *P. hysterophorus* seedlings regardless of the general assumption that most leguminous plants facilitate other plants' growth. The study highlight that biological control through competitive

plants is an approach with further potential for managing *P. hysterophorus* (Pratt *et al*., 2017) while protecting the environment from the invasive (Khan *et al*., 2013; Van der Laan *et al*., 2008). Further, despite the fact that the use of non–native plant species is not recommended for protected areas to control IAPs, these species were used in this study because they are of financial benefit to pastoralists and agro–pastoralist (Aganga & Tshwenyane, 2003; Al-Kahtani *et al*., 2017; Kariuki *et al*., 1999; Midega *et al*., 2017). This management approach may not require touching or uprooting *P. hysterophorus*, and thus, reduces health risks to people, livestock or wildlife. For countries where people, livestock and wildlife are threatened by *P. hysterophorus* such as Ethiopia (Nigatu *et al*., 2010), Pakistan (Shabbir & Bajwa, 2006), Tanzania, Kenya and Uganda (Witt *et al*., 2018), this will be a low–cost and sustainable management method for controlling the invasive, and which might promote a long–term ecosystem resilience against invasions.

While control of IAPs using suppressive forage plants have been used in other countries (Adkins & Shabbir, 2014; Khan *et al*., 2013; O'Donnell & Adkins, 2005; Shabbir *et al*., 2013), it was never tested in Tanzania. The selected species that are readily available in the country can be used in mixture to control *P. hysterophorus* and improving otherwise unusable rangelands. This control method represents a nature–friendly and effective management approach (Connolly *et al*., 2018; Knops *et al*., 1999; Ngondya *et al*., 2016a; Perkins *et al*., 2011; Schuster *et al*., 2018). If *L. purpureus* and other effective suppressive fodder species are grown together in invaded pastures this may, in addition to suppressing invasives, even promote wildlife and livestock health and production (Khan *et al*., 2013; Li *et al*., 2015; O'Donnell & Adkins, 2005; Shabbir *et al*., 2013). To effectively control *P. hysterophorus*, seeding suppressive plants must be done early before the emergence of rosettes and immediately following pulling (uprooting and burning) of mature *P. hysterophorus* seedlings prior to flowering or releasing seeds to create appropriate conditions (e.g. enough space, nutrients, water and light) for quick establishment (e.g. increase in abundance and biomass) of suppressive species. Nonetheless, selection of suppressive plants should consider native species with traits which enhance their competitive ability.

4.2.4 Effects of *Desmodium uncinatum* Leaf Crude Extract on *Parthenium hysterophorus* Growth

Another management trial to control the invasive using *D. uncinatum* leaf crude extract showed that the plant crude extract suppressed *P. hysterophorus* at various phenological

stages i.e. both seedling germination and growth, mainly under high concentrations of 75% and above. The efficient of *D. uncinatum* leaf crude extract at high concentration agrees with studies claiming that the effectiveness of bio–herbicide is dose dependent (Khaliq *et al*., 2011; Ngondya *et al*., 2016a). Interaction of natives with IAPs is affected by their non co–evolutionary background (Christina *et al*., 2015). Based on the '*novel weapon*' hypothesis, most native species are not adapted to the biochemical traits of invasive species (Callaway *et al*., 2008). The flipside of the '*novel weapon*' hypothesis or '*homeland security*' hypothesis is that within the invasive range, the plant chemistry of native or naturalized plants may be able to suppress the growth of invasive species in turn (Cummings *et al*., 2012). Since *D. uncinatum* and *P. hysterophorus* may not have co–evolved, the latter may be poorly adapted to the bio–herbicide of the former species (Christina *et al*., 2015). The study supports this '*homeland security*' hypothesis by showing that *D. uncinatum* allelochemicals exert resistance against *P. hysterophorus* seed germination and seedling growth. The results in general indicate that *P. hysterophorus* might not be adapted to the biochemical traits of *D. uncinatum* (Callaway *et al*., 2008), which makes the latter species a powerful natural tool to suppress *P. hysterophorus*.

The results show that *D. uncinatum* leaf crude extract high concentrations delayed *P. hysterophorus* seed germination. This reveals the potential to interfere early in the germination stage of *P. hysterophorus* and suppress its seeds in the soil, preventing further invasions. In addition, the growth parameters and total leaf chlorophyll content of *P. hysterophorus* seedlings were suppressed slightly under low concentrations but more strongly under high concentration treatments, which agrees with findings by Cipollini and Flint (2013), Khaliq *et al*. (2011), Namkeleja *et al*. (2013) and Ngondya *et al*. (2016b). This was likely due to *D. uncinatum* leaf extract, which has active bio–herbicidal properties as previously reported in other studies (Hooper *et al*., 2010; Khan *et al*., 2008; Ngondya *et al*., 2016b). Hooper *et al*. (2010) reported that the genus *Desmodium* can suppress *Striga hermonthica* through allelopathy when intercropped with cereals like sorghum, maize and millet. Allelochemicals of *D. uncinatum* root extract effective against the development of *S. hermonthica* include isoschaftoside, a C-glycosylflavonoid (Hooper *et al*., 2010), and uncinanone (4",5"-dihydro-2'-methoxy-5,4'-dihydroxy-5"-isopropenylfurano-(2",3";7,6)-isoflavanone) (Tsanuo *et al*., 2003). These may also be present in leaf extract and responsible for inhibition of germination and growth of *P. hysterophorus* in this study, which can help to control the invasive.

However, further studies are required to identify natural products, in particular the active components of *D. uncinatum* leaf exudate, which are suitable for large scale, low-impact deployment in *P. hysterophorus* control. The negative effect of *D. uncinatum* leaf crude extract on leaf chlorophyll content, stem height and biomass which determine seedling growth vigour suggests that the crude extract can reduce the invasive seedling's ability to photosynthesize and weakens its fitness to compete for light, nutrients, water, or other resources with resident plants (Ngondya *et al*., 2016b; Nickerson & Flory, 2015). Thus, the study results show that *D. uncinatum* leaf crude extract had the ability to weaken *P. hysterophorus* seedlings' growth. But, *P. hysterophorus* like many other IAPs may not be eradicated by using a single method, it therefore requires an integrated management approach (Khan *et al*., 2013; Shabbir *et al*., 2013; Terblanche *et al*., 2016).

Owing to its ability to regrow from broken or cut parts, and being resistant to some chemical control method, Adkins and Shabbir (2014) and Nyasembe *et al*. (2015) advised that traditional weeding and bio–herbicide approaches need to be combined with a wider strategy. For instance, management of *P. hysterophorus* using *D. uncinatum* bio–herbicide could be complemented with biological control agents such as Mexican beetle *Z. bicolorata* and suppressive plants (Shabbir *et al*., 2013), metabolites of fungal species (Javaid, 2010), or bio-herbicides from other native or naturalised allelopathic plants (Javaid, 2010; Tanveer *et al*., 2015). Feasibility of these techniques highly depend on the involvement of local communities to achieve long–term management sustainability. Also, there is a need for a coordinated national strategy for controlling biological invasions linking all management approaches (Crall *et al*., 2013). Moreover, timely detection and control of *P. hysterophorus* before it invades other habitats are essential for preventing its spreads and reducing management cost (Crall *et al*., 2013; Maistrello *et al*., 2016).

Therefore, *D. uncinatum* bio–herbicide might be utilized in sub-Saharan Africa to control *P. hysterophorus* invasion which is threatening natural and agricultural ecosystems, biodiversity management, and smallholders' livelihood. Further studies also must be carried out to confirm the safety of its leaf crude extract on native flora and fauna species to avoid suppressing beneficial plants and other living organisms.

These management approaches put forward that suppressor plants and bio–herbicide might be feasible and sustainable way of managing biological invasions of particular IAPs. Nevertheless, their management using alien species as suppressive plants may cause

significant or potentially harmful effects on the environments. This is because when alien species establish in new site tends to expands and proliferates from its original location of arrival and become invasive due to lack of or lower levels of leaf herbivory (Lake & Leishman, 2004). Their crude extracts can be used if they pose less or non-negative effects on the environment i.e. they neither kill nor suppress native flora and fauna species. People are therefore advised to avoid planting novel plants in natural areas to depress invasive plants. However, if ecologists or invasion biologists need to control the invasive species using alien plants, they must first assess and quantify their impacts at various levels of ecological complexity. They should also note that not always native species can successful suppress invasives as their competitive strength depend on abundances, and vary with life stages and along environmental gradients (Čuda *et al*., 2015). So, in order to ensure that natural areas carry out their role of biodiversity conservation and revenue creation successful, sustainable management of IAPs is inevitable.

CHAPTER FIVE

CONCLUSION AND RECOMENDATIONS

5.1 Conclusion

The study has shown that *P. hysterophorus* has invaded some villages nearby the park border zones which threaten ANP's biodiversity and ecosystem health. *Parthenium hysterophorus* invaded site had high value of CEC despite that value of Ca, Mg, K and Organic matter was lower. Control techniques used in this study suppressed seed germination and growth vigour of *P. hysterophorus*. But, these management techniques require involvement of local communities through citizen science approach to monitor *P. hysterophorus* invasion within their premises. Early detection, prompt response and eradication are considered as the most important defence in managing IAPs, and are regarded feasible in the context of protected areas species. Thus, early detection of *P. hysterophorus* within and outside ANP is significant because it can help to mitigate the invasions and reduces management cost (Crall *et al*., 2013; Maistrello *et al*., 2016). Furthermore, *P. hysterophorus* was attractive to insect flower visitors, and shares flower visitors with co–flowering plants in Northern Tanzanian semi–natural grassland habitats. *Parthenium hysterophorus* has been integrated into the plant–pollinator network, and competes for pollinators with co–flowering plant species. The results show that *P. hysterophorus*–pollinator interactions can significantly affect pollinator visitation and foraging behaviour in a recipient ecosystem and consequently affect natural plant communities. This study highlights the significance of using suppressive plant species and bio–herbicides of controlling IAPs in natural and semi–natural areas. It further supports the use of native plant species with bio–herbicide potential to reduce dependency of chemical herbicides, and promoting the use of biological herbicides.

5.2 Recommendations

The study recommends that with increasing invasion of *P. hysterophorus* in Tanzania, detailed field surveys within and outsides the border zones of protected areas and in rangelands must be conducted regularly. This could enable to identify invasion hotspots and prevent the spread of the invasive. Mechanisms and national strategies for preventing *P. hysterophorus* seed dispersal into protected areas also should be developed. Further, the study recommends that more study should be done in the following areas: modelling potential distribution of *P. hysterophorus* within and outside the border zones of ANP to help in

planning management strategies; assess whether *D. uncinatum* leaf crude can negatively impact beneficial insects i.e. decomposers, pollinators and other organisms, or suppresses native vegetation when applied in the field; and establish the shelf life and longevity of the *D. uncinatum* leaf crude extract. Furthermore, studies are required to investigate the impact of *P. hysterophorus* on wild plants' seed sets and development of co–flowering natives, and whether flower visitors transfer alien pollen of the invasive to native flowering species. Due to its complex defensive chemistry, its allelopathic effects on pollinator activity should be investigated as well.

For the countries invaded by *P. hysterophorus*, landowners may be sensitized to plant suppressive fodder species in their private lands to prevent *P. hysterophorus* invasion. In addition to deploying control measures via a community approach, the study recommends that awareness of the effects and dispersal mechanisms of *P. hysterophorus* should be raised to local communities. This might avoid accidental dispersal of *P. hysterophorus* seeds. Non–native plants were used in this study as suppressive test plant species to control *P. hysterophorus* because they can be used as forage for livestock and controlling weeds in agroecosystems. While their extracts can be considered for suppressing the invasive, one must be cautious of planting non–native plants in protected areas to control *P. hysterophorus* because they may become invasive in future. So, the study recommends the use of native plant species to suppress *P. hysterophorus*. It also advises the use of bio–herbicide because synthetic herbicides are seldom allowed in natural areas or protected rangelands for controlling IAPs.

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